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Contents

	Page
The Effect of Phthalic Acid on the Prothrombin Time of Dicumarol-treated Dogs— <i>L. B. Japans and A. P. Dunlop</i>	167
The Effects of Oxygen on the Circulatory System in Conditions of Anoxia and Asphyxia— <i>George W. Starvoiy</i>	175
The Effect of Inorganic and Organic Iodides Upon the Output of Respiratory Tract Fluid— <i>Elden M. Boyd, M. C. Blanchard, Joan Copeland, Shirley Jackson, K. Phin, and Mary Steens</i>	195
The Effect of Potassium Iodide, Sodium Iodide, and Iod-Ethamine Upon the Concentration of Alcohol-soluble and Alcohol-insoluble Fractions of Blood Iodine— <i>Elden M. Boyd and M. C. Blanchard</i>	205
Observations on Various Types of Motion Causing Vomiting in Animals— <i>R. L. Noble</i>	212
Methods of Assaying Motion Sickness Preventives on Dogs— <i>R. L. Noble</i>	226
A Human Embryo of Two to Three Pairs of Somites— <i>Ralph F. Shaner</i>	235
Resistance to Extreme Temperatures in Connection with Different Diets— <i>L. P. Dugol, C. P. Leblond, and M. Thirion</i>	244

INDEX, SECTION I, VOLUME 23.

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THE EFFECT OF PHTHALIC ACID ON THE PROTHROMBIN TIME OF DICUMAROL-TREATED DOGS¹

BY L. B. JAKES² AND A. P. DUNLOP³

Abstract

Dicumarol (10 mgm./kgm.) was administered to dogs intravenously and the prothrombin time followed. The administration of vitamin K simultaneously markedly reduced the elevation of prothrombin time following dicumarol, while the administration of large doses of sodium phthalate in the form of single injections combined with a continuous injection caused a transient lowering of the prolonged prothrombin time. This effect of sodium phthalate was not altered by removal of the kidneys. The same effect was observed in the completely eviscerated normal dog. Sodium phthalate has no effect on the prothrombin time when added *in vitro*.

Shemiakin, Schukina, and Shvezov (10) have recently suggested that vitamin K acts as the antihaemorrhagic vitamin by conversion to phthalic acid in the body and that the latter substance is the actual vitamin. The low activity of phthalic acid they explain as being due to its rapid excretion, and state that compounds such as the diethyl ester, which is presumably slowly absorbed and excreted, possess very high activity. They state that "preliminary data obtained by Kudriashev on rats with thrombinemia caused by ligating the bile duct have actually revealed the high activity of diethyl phthalate, etc." Dam (4) and Blumberg and Arnold (2) have failed to observe any antihaemorrhagic activity of either phthalic acid or diethyl phthalate in vitamin-K-deficient chicks. Karrer and Koller (6) administered sodium phthalate intravenously and diethyl phthalate orally to a case of obstructive jaundice. No improvement in the prothrombin time was observed although the intravenous injection of 1 to 2 mgm. of Synkavit restored the prothrombin time to normal in 24 hr.

Overman, Stahmann, and Link (7), using rabbits, have shown that vitamin K antagonizes the action of dicumarol. This has been confirmed clinically by Shapiro, Redish, and Campbell (9) and by Davidson and Macdonald (5). The latter showed that simultaneous administration of K and dicumarol resulted in completely preventing the hypoprothrombinemia. Brodie, Hiestand, and Jenkins (3) have confirmed the activity of vitamin K against dicumarol in rats. They also report that phthiocol, although only weakly

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Contribution from the Department of Physiology, University of Toronto, Toronto, Ont., with financial assistance from the John and Mary R. Markle Foundation.

² Assistant Professor.

³ Formerly Fellow, Department of Physiology,

antihaemorrhagic in the chick, shows about half the activity of 2-methyl-1,4-naphthoquinone when tested on the dicumarol rat. In view of Shemiakin's hypothesis, we have investigated the possible action of phthalic acid on the hypoprothrombinemia of dogs treated with dicumarol.

Methods

Dogs of 10 to 15 kgm. were used for the experiments. Where necessary, phenobarbital administered intravenously was used as the anaesthetic. We are indebted to the Charles H. Frosst Co., Montreal, for an ample supply of dicumarol. The dicumarol was dissolved in saline to give 20 mgm./cc. by the addition of a few drops of 4 *N* sodium hydroxide and was injected intravenously. The synthetic forms of vitamin K used were Synkavit (2-methyl-1,4-naphthohydroquinonediphosphoric ester, Hoffman-LaRoche) and menadione (2-methyl-1,4-naphthoquinone). Vitamin K oxide was prepared by oxidation of the menadione with hydrogen peroxide. Sources of phthalic acid were the commercial solvent diethylphthalate, administered in the form of a 1% suspension in saline, and sodium phthalate prepared by converting potassium diphthalate to the neutral sodium salt. Plasma prothrombin times were determined according to Quick (8), but using 0.1 *M* calcium concentration. Blood prothrombin times were measured by adding 0.2 cc. of blood to 0.1 cc. of plasma. The thromboplastin used in both determinations was prepared from acetone extracted rabbit brain by heating 0.5 gm. of dried brain in 10 cc. of saline at 56° C. for 15 min. and taking the supernatant suspension.

Results

Effect of Vitamin K on the Action of Dicumarol in the Dog

The observation of the antagonizing effect of vitamin K on the action of dicumarol has been reported in rabbits and humans but there have been no reports of this effect in the dog. A series of dogs were given 10 mgm./kgm. of dicumarol simultaneously with varying doses of vitamin K or phthalic acid. The plasma prothrombin times were followed daily. As a control on each dog, dicumarol was also administered without vitamin K.

As shown in Fig. 1a, 10 mgm./kgm. of dicumarol raised the prothrombin time to 7 min. by the fourth day, while with the simultaneous administration of 10 mgm./kgm. of vitamin K oxide, the prothrombin time rose only to 1 min., 45 sec., this value being reached on the third day. Without vitamin K oxide the prothrombin time did not return to normal until the 10th day and the administration of vitamin K oxide brought it back to normal by the fifth day.

This effect was consistently obtained. While not sufficient animals were studied to establish a relationship between the dosage of dicumarol and the dosage of vitamin K necessary to neutralize the effect of the dicumarol, our experiments suggested that the dose of vitamin K required was of the same order as the dose of dicumarol given, but varied with the form of vitamin K

used. Thus 1.75 mgm./kgm. of vitamin K oxide had no effect on the dicumarol curve. The peak prothrombin time found was 3 min., 38 sec. on the fourth day (peaks in control experiments 3 min., 40 sec. on the fifth day, 11 min., 22 sec. on the fourth day). However, 0.75 mgm./kgm. of Synkavit did have an effect on this animal after dicumarol. The action of the dicumarol was shortened, the peak prothrombin time of 2 min., 20 sec. being reached on the second day.

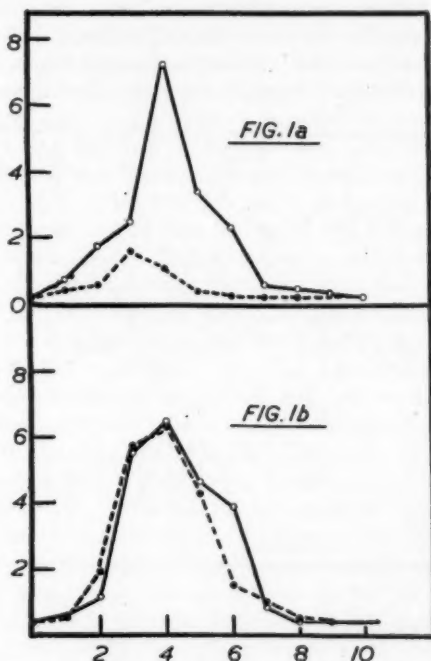


FIG. 1. Effect of vitamin K and phthalic acid on the plasma prothrombin time following dicumarol; 10 mgm./kgm. of dicumarol given intravenously at zero time. (a) ●—● 10 mgm./kgm. of vitamin K oxide given simultaneously. (b) ●—● 100 mgm./kgm. of sodium phthalate given intravenously immediately, 50 mgm./kgm. of diethyl phthalate daily subcutaneously for four days.

In Fig. 1b are shown the results obtained when sodium phthalate was administered. A single dose of 100 mgm./kgm. of sodium phthalate was given simultaneously with the dicumarol, followed by daily subcutaneous injections of 50 mgm./kgm. of diethyl phthalate for four days. The results are shown in Fig. 1b and indicate that phthalic acid had no effect on the action of dicumarol, the peak being reached by the fifth day with and without phthalic acid and the peak value being 6 min., 30 sec. and 6 min., 20 sec.; also in both cases the prothrombin time returned to normal about the eighth day. Another dog was given a continuous injection of sodium phthalate at a rate of 10

mgm./kgm./hr. for 48 hr.; dicumarol was administered when the phthalate injection was started. Here again, the phthalic acid did not affect the action of dicumarol, the prothrombin time on the third day without phthalic acid being 1 min., 17 sec. and with phthalic acid being 1 min., 50 sec.

The Effect of Continuous Injections of Phthalate on the Blood Prothrombin Time of the Dicumarol-treated Dog

In a further series of experiments, dogs were given 5 or 10 mgm./kgm. of dicumarol 48 hr. before the experiment. They were anaesthetized, the jugular vein cannulated for injection of the phthalate, and the femoral veins exposed to obtain blood samples. A continuous injection of sodium phthalate was established and blood prothrombin times determined at 10-min. intervals

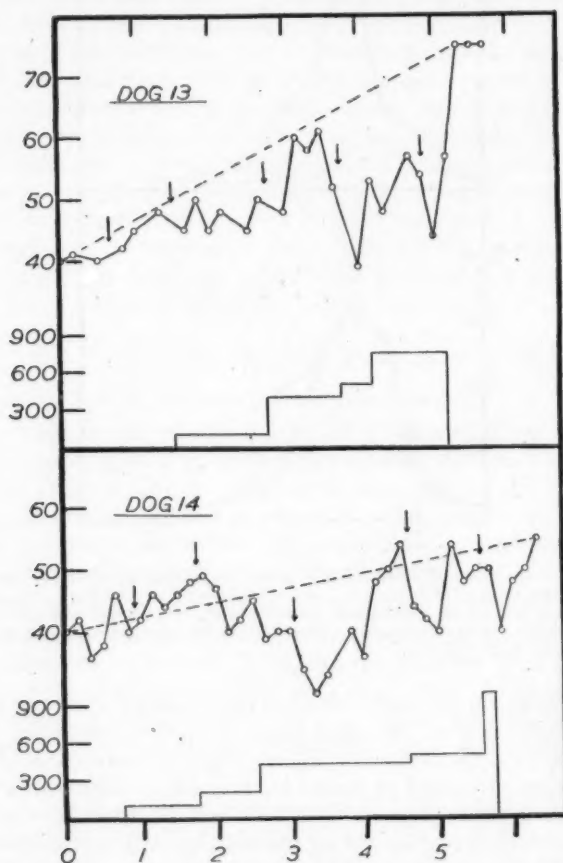


FIG. 2. Effect of sodium phthalate on the blood prothrombin time of the dicumarol-treated dog. Continuous injection of sodium phthalate shown in mgm./hr. \downarrow Single intravenous injections of 500 mgm. of sodium phthalate.

for a period of five to six hours. Fig. 2 shows the results obtained from the continuous injection of sodium phthalate plus single injections of 500 mgm. of sodium phthalate administered intravenously in 10 cc. of saline. In dog No. 13 the blood prothrombin time was still rising from the effect of the dicumarol. At the beginning of the experiment it was 40 sec. and had reached 75 sec. in six hours. Therefore the change in prothrombin time during the experiment should approximately follow the dotted line. A single injection of sodium phthalate was given and had little or no effect on the blood prothrombin time, which continued to rise to 48 sec. A continuous injection of sodium phthalate of 100 mgm./hr. was then started and a single injection of 500 mgm. given simultaneously; this brought the blood prothrombin time down to 45 sec. and kept it at this level, between 45 sec. and 50 sec., for an hour and a half. The blood prothrombin time then began to rise in spite of the continuous injection being increased to 400 mgm./hr. plus a second single dose of 500 mgm. Another 500 mgm. plus a further increase in the rate of continuous injection was then given and this brought the prothrombin time down to the 48 sec. level. The continuous injection was further increased and this kept it at approximately the same level for 75 min. Then the continuous injection was stopped and the blood prothrombin time rose immediately to 75 sec. where it stayed for the duration of the experiment. At the beginning of the experiment, dog No. 14, also shown in Fig. 2, had a blood prothrombin time of 40 sec. which had risen to 55 sec. at the end of six hours. A single injection plus a continuous injection of 100 mgm./hr. had no effect on the blood prothrombin time, which rose to 48 sec. An increase in the continuous injection to 180 mgm./hr. plus a single injection brought the time down to 40 sec. It began to rise again (45 sec.) and was brought back to 40 sec. value by increasing the continuous injection to 400 mgm./hr. Another single injection produced a fall in prothrombin time down to 30 sec. The prothrombin time then rose again and reached 54 sec. an hour later. The continuous injection was then increased to 500 mgm./hr. and another single injection given, which brought it down to 40 sec. Then the prothrombin time rose to 50 sec. This was decreased again to the 40 sec. value by a single injection together with a continuous injection of 1000 mgm./hr. The continuous injection was then stopped and the prothrombin time rose to 55 sec.

Five dogs have been subjected to this procedure and the same effect of phthalate on the blood prothrombin time has been observed consistently in all five. The effect of phthalate was greatest in those animals with the longest prothrombin time. Another dicumarol-treated dog was not given a continuous injection of disodium phthalate but only large single doses. In this animal the prothrombin time fell after the first injection, but no effect was produced by further doses. In general the effect was only seen if both single and continuous injections were combined.

A similar series of experiments were carried out in which diethyl phthalate was used instead of sodium phthalate. Diethyl phthalate was administered

as single injections of 50 mgm. at hourly intervals. There was no definite change in the blood prothrombin time over the period of observations. In five dogs of this series both kidneys were removed before administration of diethyl phthalate. These likewise showed no change in the prothrombin time.

The Effect of Phthalic Acid in Vitro

As shown above, a suitable injection of phthalic acid causes a transient lowering of the blood prothrombin time. To test the effect of phthalate *in vitro*, varying quantities of sodium phthalate were added to the thromboplastin, and the blood prothrombin time determined. The results are shown in Table I. The phthalic acid did not shorten the blood prothrombin time *in vitro*.

TABLE I
EFFECT OF SODIUM PHTHALATE ON THE PROTHROMBIN TIME *in vitro* OF BLOOD FROM DICUMAROL-TREATED DOGS

Concentration of sodium phthalate, mgm./cc.	Prothrombin time				
	Dog 1		Dog 2		Dog 3
	Min.	Sec.	Min.	Sec.	Sec.
0	2	40	3	20	20
0.035	3	5	3	50	18
0.07	2	55	3	44	22
0.175	3	0	4	22	21
0.35	3	3	4	22	20
0.7	3	0	4	55	27

Effect of Nephrectomy and Evisceration on the Effect of Phthalate

In two dogs both kidneys were removed immediately before the dogs were subjected to the same experiment as Dogs 13 and 14 (Fig. 2). Fig. 3a shows the results obtained from one of these dogs. After nephrectomy the blood prothrombin time was 2 min., 30 sec., which rose to 3 min., 30 sec. in the next 30 min. A single injection and a continuous injection of 300 mgm./hr. brought this time down to 2 min., 50 sec. in 20 min. Then the blood prothrombin time rose to 3 min., 50 sec. in the next 30 min. but was decreased to 3 min., 0 sec. at one hour, 10 min. by another single injection with a continuous injection of 200 mgm./hr. In another half an hour it had risen again to 4 min., 5 sec., this time being reduced to 3 min., 25 sec. in 10 min. by an increase in the continuous injection up to 425 mgm./hr. along with another single injection. In 20 min. it rose again to 4 min., 20 sec. and was then decreased to 3 min., 5 sec. in 10 min. by another single injection with the continuous injection reduced to 250 mgm./hr. Then it rose again in 20 min. to 4 min., 10 sec. and the continuous injection was stopped. The other dog gave similar results.

In a further series of dogs that were not injected with dicumarol previous to the experiment, a complete evisceration was performed, all the abdominal

viscera except the kidneys and adrenals being removed. After completion of the operation the effect of phthalic acid on the blood prothrombin time was tested as before; 250 mgm./kgm. of glucose were given intravenously at hourly intervals. The results obtained with one of these dogs are shown

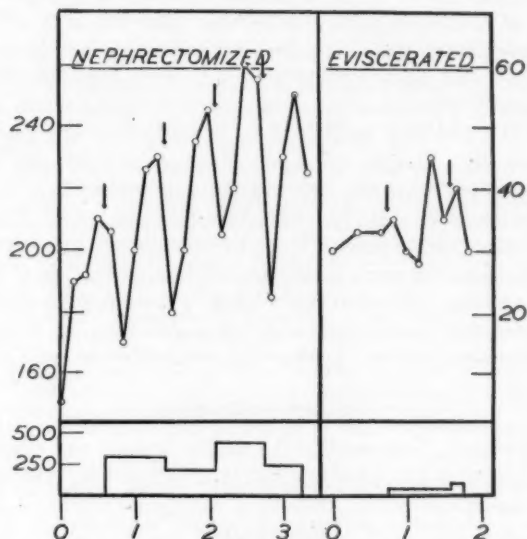


FIG. 3. Effect of sodium phthalate on the blood prothrombin time of the dicumarol-treated dog. Continuous injection of sodium phthalate shown in mgm./hr. ↓ Single intravenous injections of 500 mgm. of sodium phthalate.

in Fig. 3b. After evisceration the blood prothrombin time was 30 sec. and rose to 33 sec. in 20 min. Then a single injection of phthalate was given and a continuous injection of 100 mgm./hr. was started, which brought the time down to 28 sec. A second single injection together with an increase in the continuous injection to 200 mgm./hr. brought the prothrombin time down from 35 sec. to 30 sec. Evisceration was attempted on dicumarol-treated animals, but these animals did not survive for a sufficient time to test the effect of phthalate.

Discussion

As shown above, vitamin K in large doses neutralizes the action of dicumarol in the dog. The injection of phthalic acid will also lower the prothrombin time of the dicumarol-treated dog. However, this action does not appear to be a vitamin-K-like effect. The action is very transient. Thus, on the average, the prothrombin time was depressed only for periods of 30 to 45 min. after the injection. Shemiakin, Schukina, and Shvezov explain the fleeting action of phthalate as being due to rapid excretion in the urine. However, this does not explain the transient nature of the action of phthalate observed,

since removal of the kidneys did not prolong it. Diethyl phthalate was found by them to be more effective and as this would maintain a concentration of phthalate in the body for a longer time, they conclude it to be further evidence in favour of their hypothesis. However, diethyl phthalate was not found to exert any effect on prothrombin time in our experiments. Again, the lowering of the prothrombin time was observed only with very high dosages of phthalic acid and required a continuous injection of phthalate simultaneously. The dosages of phthalic acid were considerably greater than those of vitamin K required in the same animal. If vitamin K acted through conversion to phthalic acid, smaller doses of phthalic acid should be required than of vitamin K. Finally, the same effect of phthalic acid was observed on the prolonged prothrombin time following evisceration. This is in contrast to the action of vitamin K, since Andrus, Lord, and Moore (1) showed that K had no effect after removal of the liver, while it suggests that the action of phthalate is exerted through a response of tissues other than the abdominal viscera. It is evident therefore that while a lowering of the prothrombin time by the injection of phthalic acid had been observed in the dicumarol-treated dog, this effect does not appear to be related to the action of vitamin K.

Acknowledgments

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THE EFFECTS OF OXYGEN ON THE CIRCULATORY SYSTEM IN CONDITIONS OF ANOXIA AND ASPHYXIA¹

BY GEORGE W. STAVRAKY²

Abstract

An analysis is presented of the blood pressure changes during anoxia, asphyxia, and oxygen administration, in 34 animal experiments. Similarly, in 30 human beings during decompression equivalent to altitudes ranging from 16,500 ft. to 29,000 ft., the blood pressure findings are correlated with the action of the heart and the state of the peripheral blood vessels, and the effect of subsequent administration of oxygen upon them is investigated.

Sudden deprivation of oxygen leads to a vasoconstrictor response, which, in humans, manifests itself in facial pallor and elevation of the blood pressure. The administration of oxygen in the later stages of this response may produce a further transient elevation of the blood pressure, which is followed by a fall of blood pressure and slowing of the pulse. The rise of blood pressure caused by oxygen after a period of acute anoxia or asphyxia is due to an augmentation of the action of the heart and to an intensification of the vascular tone, the two phenomena contributing to the rise of blood pressure in a varying degree under different experimental conditions. In intact, anaesthetized cats the effect persists after adrenalectomy. In spinal preparations, previously kept on "minimal" respiration, the effect is greatly reduced by the removal of the suprarenal glands. The rise of blood pressure resulting from the administration of oxygen is abolished by the destruction of the spinal cord by pithing, and is therefore attributed to an excitation of the sympathetic centres. Evidence also is presented that suggests that the chemoreceptors participate in this response in intact anaesthetized animals.

A protracted oxygen deficiency of a moderate degree leads to a vasodilator reaction. In human subjects it manifests itself in a gradual engorgement of the cutaneous blood vessels, often in a lowering of the blood pressure, and an increase of the pulse rate. Sudden administration of excessive quantities of oxygen under these conditions causes a further decline of blood pressure and a slowing of the pulse. An analysis of the fall of blood pressure caused by the administration of oxygen in conditions of prolonged hypo-oxygenation shows that it is not strictly related to changes in respiration or to acapnia, which occurs during breathing of air deficient in oxygen. Neither is it prevented by addition of carbon dioxide to the oxygen. However, under prevailing experimental conditions, this fall of blood pressure is almost invariably abolished by a bilateral vagotomy, is occasionally reduced by atropine, and is absent in spinal preparations, these observations indicating that it is dependent on the functioning of the medullary reflex mechanisms.

Introduction

The effects of anoxia and asphyxia on the circulatory system are well known, having been investigated by means of a variety of methods. However, the complexity of the mechanism involved in the regulation of the circulation, and the effects that changes in aeration have on the nervous

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² Associate Professor of Physiology.

system as well as on the circulatory system itself, make the study a difficult one, and a survey of the literature shows that a considerable divergence of opinion is encountered in the interpretation of the results. (A bibliography and reviews of literature on the various aspects of the subject are to be found in publications by Hoff and Fulton (13, pp. 30-42), Gellhorn and Lambert (8, pp. 11-71), Tavel (31), Van Liere (32, pp. 73, 108, 222), Opitz and Tilmann (19-22), Wiggers (34), Brazier (6), Schmidt and Comroe (23, 24), Gesell (9, 10 (p. 221)), and others.)

In contrast to the study of oxygen-want, the effects of the administration of oxygen on the circulatory system in various degrees of anoxia and asphyxia have received comparatively little attention, and only isolated observations can be found regarding this subject. Since the early work on asphyxia and anoxia (15, 17, 18, 29 (Vol. 2, p. 307), etc.) it is known that following the interruption of the oxygen supply in experimental animals, resumption of oxygenation usually leads to a rise in blood pressure superimposed on the rise that occurs as a result of the anoxia or the asphyxia itself. Kaya and Starling (15) attribute this secondary rise of blood pressure to the revival by oxygen of the power of the contractions of the heart. Mathison (18) mentions in addition a possible revival by oxygen of the vasomotor centre. Likewise Opitz and Tilmann (20) in their recent study of the circulation in conditions of low barometric pressure refer to a phase of "post-hypoxaemic excitation of the sympathetic nervous system," which takes place during the descent of animals from high altitudes in a decompression chamber.

In observations on human subjects the postanoxic rise in blood pressure was occasionally noted by Besserer (4). On the other hand, Schwarz and Malikiosis (28) and Schwarz (27) have found that oxygen administered after a period of hypoxia caused circulatory embarrassment, which manifested itself in a fall of blood pressure and slowing of the heart; such effects were associated with an acute feeling of discomfort, dizziness, fainting, and even convulsive manifestations. Schubert (26) has observed that similar declines in blood pressure followed by convulsions were prone to occur in experimental animals during sudden returns from low barometric pressures to ground levels, while Bean and Whitehorn (3) have found, in studies on decerebrate dogs exposed to the action of oxygen under a pressure of five atmospheres, that during the early stages of oxygen-poisoning there was a resultant bradycardia that was abolished by vagotomy, though the terminal effects in the same direction were not dependent upon the vagi nerves. In contradistinction to these observations Willmon and Behnke (35) have noted in human beings that inhalation of oxygen under a pressure of four atmospheres produced vasoconstriction.

In view of the practical importance of the subject, the effects of the administration of oxygen on the circulatory system in conditions of oxygen-want were reinvestigated and the results obtained during this study are presented here.

Methods

Both animals and human beings were employed as subjects during this investigation. Dogs, cats, and rabbits, anaesthetized with chloralose, urethane, or dial as well as decerebrate and spinal preparations, were used in a series of 34 experiments. Asphyxia was produced by clamping of the tracheal cannula or by discontinuing artificial respiration. A condition of anoxia was obtained by substituting nitrogen or a mixture low in oxygen for the inspired air. Nitrogen and oxygen were administered from a Douglas bag (7) through specially constructed delicate valves. The blood pressure was recorded from the carotid or femoral artery by means of a mercury manometer; in a few experiments this was paralleled by cardiometric recordings or by plethysmographic estimation of the volume of a loop of intestine. In control experiments in which adrenalectomy was performed this was carried out in the following manner: at the desired stage of the experiment the recordings were temporarily discontinued, the peritoneal cavity was opened in the mid-line, the suprarenal veins were ligated, the glands removed, and the experiment continued as soon as the blood pressure of the animal became stabilized.

In 30 trained human subjects, anoxia was produced by reducing the pressure of the inspired air in a low pressure chamber. A gradual anoxia was induced by a slow reduction of pressure in the chamber without addition of oxygen. Short periods of acute anoxia were brought about in the following manner: the tested subjects, inhaling oxygen supplied by means of a Boothby-Lovelace-Bulbulian mask (5) were taken up to a given altitude, then the mask was removed for a given interval. In a number of experiments pure oxygen was administered from a Douglas bag (7) instead of through a mask and flow metre. In human subjects continuous readings of the blood pressure were made from the brachial artery by the auscultatory method; the pulse was counted and recorded at definite intervals.

Results

EFFECTS OF AERATION AND OXYGEN ADMINISTRATION IN CONDITIONS OF OXYGEN-WANT AS STUDIED IN EXPERIMENTAL ANIMALS

Part I—Effects of Resumption of Aeration or Oxygen Administration Following Rapidly Induced Asphyxia and Anoxia

In accordance with other investigations, it was found in the present series of experiments, that sudden asphyxia induced in previously adequately aerated animals led to a progressive rise in blood pressure. In addition to this asphyxial elevation of the blood pressure, in the majority of the experiments resumption of aeration was followed by a further rise in blood pressure, the magnitude of the response varying in individual animals and in different forms of experiments from a few millimetres of mercury to as much as 130 mm. Hg. This secondary rise of blood pressure was usually more marked after prolonged periods of asphyxia but never occurred during the asphyxia

itself, even when the animal was allowed to die from suffocation (Fig. 1, A and B). Similar results were obtained by substituting periods of administration of nitrogen for suffocation, though as noted by Mathison (18), in

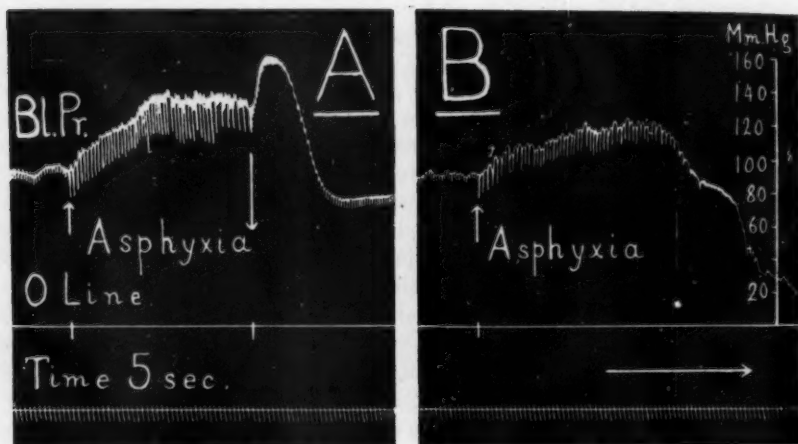


FIG. 1. Effects of asphyxia induced by means of clamping the tracheal cannula in a rabbit under urethane anaesthesia.

Note the secondary rise of blood pressure, which appeared on resumption of aeration in tracing A. This secondary rise of blood pressure was absent when asphyxia was continued until the death of the animal as shown in tracing B.

intact animals the breathing of nitrogen frequently resulted, after an initial rise in blood pressure, in a sudden fall of the latter and a slowing of the heart, this response being particularly prominent in the cat (Fig. 2 A).

In the case of administration of oxygen, after inhalation of nitrogen, or after suffocation, the results were essentially the same as in the case of resumption of aeration; however, occasionally a slight fall in blood pressure followed the secondary rise before the blood pressure attained a constant level (Fig. 2 A). If the preceding inhalations of nitrogen were of very short duration, only a slight fall in blood pressure or a return to the normal level was seen to ensue on oxygen administration.

The rise in blood pressure that took place on resumption of oxygenation following a sufficiently severe period of asphyxia or anoxia was not associated with any motor manifestations and, as determined in spinal preparations, was accompanied by an acceleration of the heart, an increase in the stroke volume of the ventricles, and a reduction in the diastolic volume of the heart, which was increased during the asphyxia or inhalation of nitrogen. In control experiments in which plethysmographic recordings of a loop of intestine were carried out, it was occasionally found that the initial phase of the administration of oxygen or resumption of aeration coincided with a reduction in the volume of the intestine, followed by an increase in its volume. The

vasoconstriction thus determined was particularly prominent in experimental conditions in which asphyxia or anoxia by itself did not cause a rise of blood pressure, this being in accordance with the observation of Mathison (18 (pp. 290, 291) and Fig. 8).

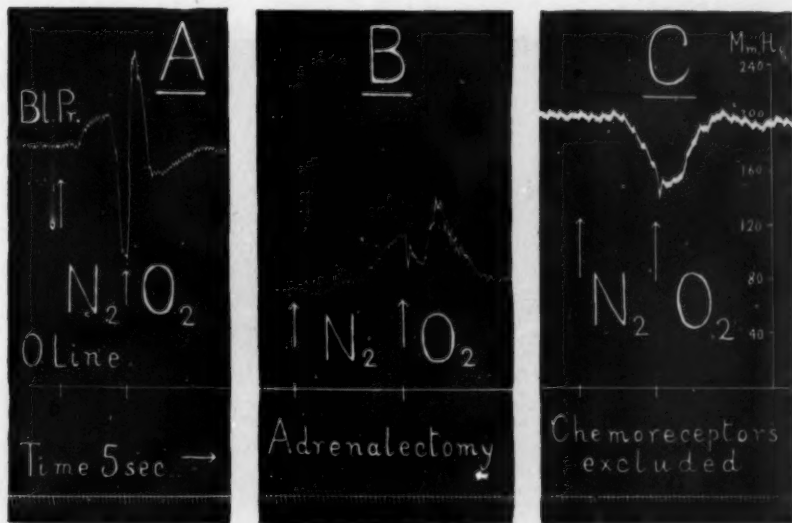


FIG. 2. Effects of oxygen after abruptly induced periods of anoxia in three different cats under chloralose and urethane anaesthesia (65 to 75 mgm. chloralose and 195 to 210 mgm. urethane per kgm. body weight).

A: Inhalation of nitrogen followed by oxygen in an intact cat.

B: Inhalation of nitrogen followed by oxygen after adrenalectomy. Note on administration of oxygen a sudden termination of the fall of blood pressure followed by a secondary rise of the same.

C: Inhalation of nitrogen followed by oxygen after sectioning of the vagi nerves and denervation and exclusion between ligatures of the carotid sinuses. Note absence of any elevation of the blood pressure above the resting level either during nitrogen or oxygen administration in an anaesthetized animal. (This tracing should be compared with those of Mathison (18) in which both administration of nitrogen and subsequent resumption of aeration resulted in rises of blood pressure in spinal preparations).

The effects of oxygen after a period of acute anoxia or asphyxia were essentially the same in intact anaesthetized animals and in decerebrate and spinal preparations. In anaesthetized animals with completely excluded chemoreceptors, however, upon inhalation of nitrogen, a progressive fall in blood pressure occurred, and the administration of oxygen at this time led only to a restoration of the blood pressure to its previous level (Fig. 2 C).

Control experiments were carried out in order to ascertain whether the rise of blood pressure that took place on resumption of aeration or oxygen administration depended on a purely nervous mechanism or whether it was due to the liberation of adrenaline or both. As can be seen in Fig. 2 B in an intact anaesthetized cat, the rise of blood pressure that took place on resump-

tion of aeration was quite prominent after the removal of the suprarenal glands. However, in spinal preparations that were placed on a "minimal ventilation"*, adrenalectomy greatly reduced the effects of oxygen and only slight residual signs of excitation of the sympathetic nervous system remained after the operation (Fig. 3 B).

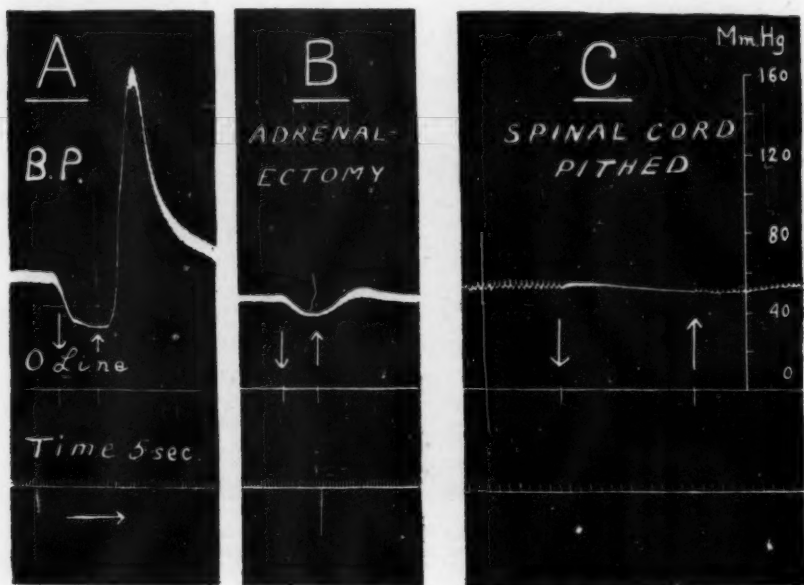


FIG. 3. Analysis of the postasphyxial rise of blood pressure in a spinal cat after a prolonged period of "minimal ventilation".

A: Interruption of artificial respiration for 60 sec. resulted in a drop of blood pressure instead of a rise. The postasphyxial rise of blood pressure on resumption of ventilation was very marked.

B: Reduction of the postasphyxial rise of blood pressure after adrenalectomy. (In all these experiments the animals were killed by suffocation during which no rise of blood pressure ensued until the death of the animal).

C: Control experiment on a cat in which pithing of the spinal cord completely abolished both the asphyxial fall of blood pressure and the postasphyxial rise of the same. (In this experiment the suprarenal glands were intact and the usual response (as in Fig. 3 A) was obtained before pithing of the cord).

In experiments in which "minimal ventilation" was induced, it was found that acute asphyxia by itself did not lead to rises in blood pressure. As shown in Fig. 3 A, when a spinal cat was kept on a "minimal ventilation" an interruption of aeration caused a fall, instead of a rise, in blood pressure. Cardiometric recordings showed that this fall in blood pressure coincided with the usual dilatation of the ventricles, but was not accompanied by an acceleration of the heart or an increase of the stroke volume as commonly seen when

* The terms "minimal" and "optimal ventilation" were defined in a previous paper (30).

asphyxia was induced after "optimal" aeration. Motor manifestations were likewise absent, no convulsions occurring during the interval of suffocation. When the animal was placed on "optimal ventilation" recovery took place, and asphyxia again caused a rise in blood pressure and augmentation of the contractions of the heart (Fig. 4, A and B). It may be noted that in order for asphyxia to produce an acceleration of the heart and convulsive manifestations, longer periods of recovery on "optimal ventilation" were required than were necessary for the production of a rise in blood pressure or an increase of the stroke volume of the ventricles.

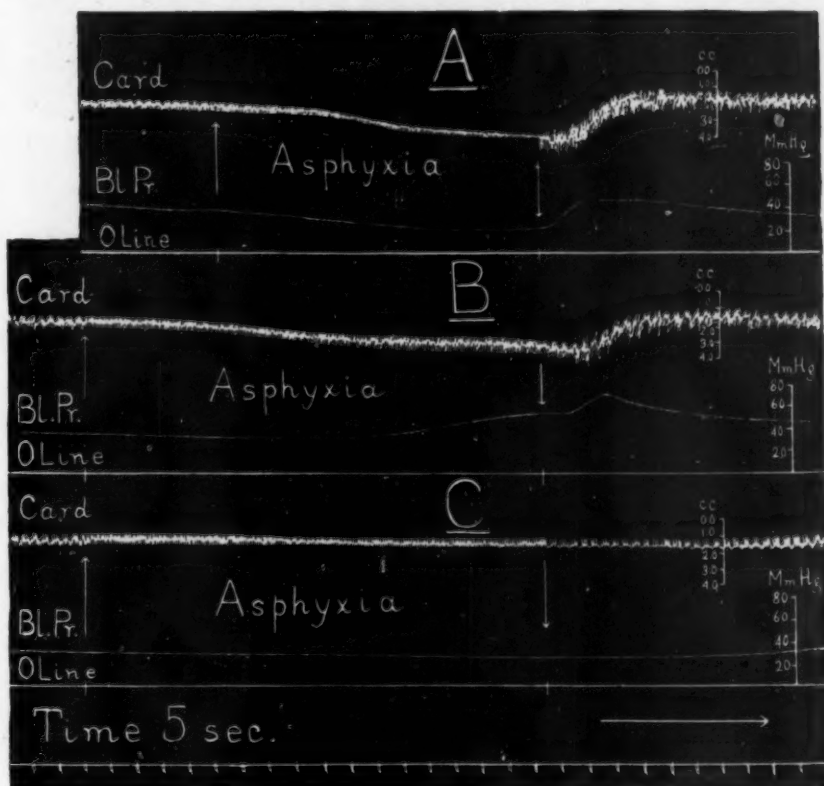


FIG. 4. Blood pressure tracings and cardiometric recordings during asphyxia and during resumption of aeration in a spinal cat. (In the cardiometric tracings upstroke represents systole).

A: Effect of interruption of artificial respiration after a period of "minimal ventilation." On resumption of respiration "optimal ventilation" was induced and this was continued until the end of the experiment.

B: Effect of interruption and resumption of artificial respiration after a period of "optimal ventilation."

C: Effect of interruption and resumption of artificial respiration after pithing of the spinal cord.

Provided that the spinal cord was intact in the experimental animal, a resumption of aeration or administration of oxygen following a period of asphyxia invariably caused a marked rise of blood pressure, an acceleration of the heart, and an increase in the stroke volume of the ventricles and reduction in their diastolic volume, irrespective of the previous response to asphyxia (Fig. 4, A and B).

After complete destruction of the spinal cord by pithing, asphyxia caused a progressive slowing of the heart and reduction of the stroke volume of the ventricles, such changes being gradual and not accompanied by a significant lowering of blood pressure. These effects were independent of the preceding degree of ventilation of the preparation, and resumption of aeration or administration of oxygen after a period of asphyxiation resulted in a slow restoration of the action of the heart followed by a supernormal phase, which was expressed by a very slight rise of blood pressure (Fig. 3 C and 4 C).

Part II—Effects of Oxygen Administration After Prolonged Oxygen Deficiency

The effects of administration of oxygen following periods of prolonged

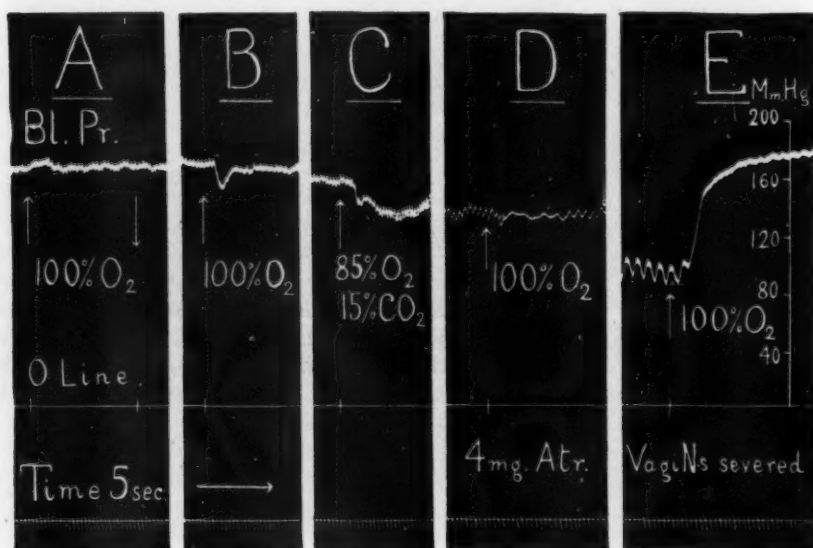


FIG. 5. Effects of oxygen after gradually induced anoxia in a cat under chloralose and urethane anaesthesia (1:3).

A: Effect of substituting 100% oxygen for normal air in a spontaneously breathing animal.

B: Effect of administration of 100% oxygen after the animal breathed a mixture of 9% oxygen and 91% nitrogen for 12 min.

C: Same procedure as in B repeated with 85% oxygen and 15% carbon dioxide.

D: Same procedure as in B repeated after an intravenous injection of 4 mgm. of atropine sulphate. (Atropine was injected three minutes before the substitution of 9% oxygen for air, or 15 min. before the administration of 100% oxygen).

E: Same procedure as in B repeated after sectioning of the vagi nerves in the neck.

oxygen-lack were quite different from those prevailing after a brief interval of acute anoxia or asphyxia.

Administration of 100% oxygen to a spontaneously breathing animal under chloralose and urethane anaesthesia (50 to 75 mgm. chloralose, and 150 to 225 mgm. urethane per kgm. body weight) had no effect on the level of the blood pressure when the animal had been previously breathing air, only the respiratory rate somewhat decreasing. As shown in Figs. 5 and 6 in the cat,

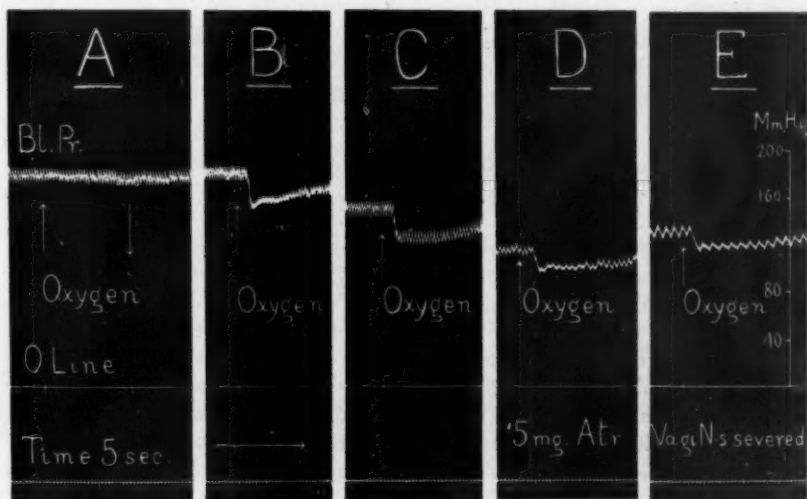


FIG. 6. Effects of oxygen after gradually induced anoxia in a cat under chloralose and urethane anaesthesia (1:3).

A: Effect of substituting 100% oxygen for normal air in a spontaneously breathing animal.

B: Effect of administration of 100% oxygen after the animal breathed a mixture of 9% oxygen and 91% nitrogen for 15 min.

C: Effect of administration of 100% oxygen after the animal breathed a mixture of 9% oxygen, 5% carbon dioxide, and 86% nitrogen for 15 min.

D: Same procedure as in B repeated five minutes after the intravenous injection of 5 mgm. of atropine sulphate.

E: Same procedure as in B repeated after sectioning of the vagi nerves in the neck.

which was found to be most suitable for these experiments, a substitution of air by a mixture of 9% oxygen and 91% nitrogen for a period of 12 to 15 min. caused some increase in the depth of respiration, but usually left the blood pressure almost unaffected. In 10 out of 11 experiments, the blood pressure rose slightly in the first five minutes, returning to its normal level toward the end of a 12 to 15 min. period of inhalation of 9% oxygen. However, the administration of 100% oxygen then caused a fall of blood pressure, a slowing of the heart, and generally a marked decrease in the volume and rate of respiration. The decline of blood pressure amounted to as much as 40 mm. Hg, a value that equalled 20 to 25% of the total blood pressure. One cat seemed

unable to maintain its blood pressure on mixtures deficient in oxygen; in this animal the subsequent administration of 100% oxygen yielded no pronounced decline in the blood pressure level.

In two dogs, breathing with 9% oxygen caused a much more pronounced hyperpnoea and a more sustained elevation of blood pressure than in the cat, the administration of 100% oxygen at this time resulting also in apnoea, a decline of blood pressure, and a slowing of the heart, the latter reaction being more pronounced than that taking place in the cat.

An analysis of the mechanism of the fall of blood pressure produced by oxygen was attempted, and in spite of the considerable variability of the results, a description of these experiments will be given.

Effect of Carbon Dioxide

The addition of carbon dioxide to the mixture of gases used showed that the fall of blood pressure caused by oxygen administered after periods of hypoxia was not necessarily associated with changes in the carbon dioxide content of the blood. In order to illustrate this, two representative experiments will be quoted. In one, after the animal inhaled 9% oxygen in nitrogen for 12 min., a mixture of 85% oxygen and 15% carbon dioxide was administered. As shown in Fig. 5 C the addition of 15% carbon dioxide to the oxygen did not prevent the fall of blood pressure or cessation of respiration. In some experiments, the initial fall of blood pressure and the slowing of the heart caused by oxygen were less marked, but the total period of depression of the circulation was usually prolonged by the addition of carbon dioxide to the oxygen.

In another form of experiment, in order to prevent blowing off of carbon dioxide during the 15 min. period of hypo-oxygenation, 5% carbon dioxide was added to the mixture of oxygen and nitrogen, the mixture consisting of 9% oxygen, 5% carbon dioxide, and 86% nitrogen. The addition of carbon dioxide to the mixture markedly increased the over-breathing as compared to a control period of inhalation of 9% oxygen in nitrogen, and the excitation of the respiratory centre by this mixture of gases was so severe that it counteracted the depression of respiration that usually occurred on administration of oxygen following a period of hypoxia. However, the depressing effect of oxygen on the circulatory system persisted, a prompt decline of the blood pressure ensuing in response to the administration of oxygen (Fig. 6 C).

Effects of Sectioning of the Vagi Nerves and Exclusion of the Carotid Sinuses

In nine experiments in which periods of hypo-oxygenation were followed by administration of oxygen, the vagi nerves were divided. After sectioning of the vagi nerves, the blood pressure frequently lost some of its stability and 12 to 15 min. of inhaling 9% oxygen resulted in an appreciable lowering of the blood pressure level. In eight out of nine of the experiments following the sectioning of the vagi nerves, administration of oxygen after a period of breathing with a 9% oxygen mixture caused a rise instead of a fall in blood pressure (Fig. 4 E), though in one of these experiments, a transient fall of blood pressure preceded the rise. In one experiment, however, the fall of

blood pressure caused by oxygen persisted after the sectioning of the vagi nerves (Fig. 6 E). In this experiment, in addition to the sectioning of the vagi nerves, a complete denervation and exclusion between ligatures of the carotid sinuses was carried out. After this operation, the cat could not breathe 9% oxygen for more than three or four minutes at a time, a profound lowering of the blood pressure taking place. Administration of oxygen restored the blood pressure to its previous level. This experiment of sectioning of the vagi nerves and complete exclusion of the carotid sinuses was repeated on two other occasions with identical results—the animals not being able to withstand breathing 9% oxygen for any length of time.

On the whole, the reaction of the animals to hypo-oxygenation after sectioning of the vagi nerves and complete exclusion of the carotid sinuses became similar to that of spinal preparations in which any reduction of ventilation below the "optimal level" lowered the blood pressure, whereas an increase of the ventilation from the "minimal" to the "optimal" level raised it again (30).

Inasmuch as after sectioning of the vagi nerves the inhalation of 9% oxygen usually reduced the level of the blood pressure, an experiment was performed in order to eliminate the possibility that the lowered blood pressure itself was responsible for the change in the reaction to oxygen. In a cat, sectioning of the vagi nerves was combined with ligation of the common carotid arteries. This incomplete exclusion of the carotid sinuses raised the blood pressure to 315 mm. Hg yet breathing of a 9% oxygen mixture was well tolerated by the animal, the blood pressure at the end of a 15 min. period being lowered only to the level prevailing at the beginning of the experiment. In spite of this high blood pressure level, administration of oxygen now caused a rise of blood pressure instead of a fall.

Effect of Atropine

Finally, in an attempt to determine whether sectioning of the vagi nerves interfered with the centripetal or centrifugal fibres, atropine sulphate was injected intravenously in quantities of 1 to 2 mgm. per kgm. body weight in eight experiments. In two experiments, atropinization precluded the fall of blood pressure caused by oxygen. In one of these experiments, the injection of atropine that was carried out during the breathing of 9% oxygen caused a considerable lowering of the blood pressure and a reversal of the effect of the subsequent oxygen administration. In the other experiment, atropine was injected before the inhalation of the 9% oxygen mixture, but it still precluded the fall of blood pressure caused by oxygen (Fig. 5 D). In both experiments the effect of atropine was not lasting, and after 20 to 30 min. the usual reaction to oxygen was restored. In the remaining six experiments, otherwise complete atropinization did not abolish the effect of oxygen, though in several experiments atropine seemed to reduce somewhat the fall of blood pressure caused by oxygen (Fig. 6 D). It so happened that atropine and sectioning of the vagi nerves respectively abolished and reversed the fall of blood pressure caused by oxygen in the experiment shown in Fig. 5, but both had no effect

in the experiment shown in Fig. 6. Usually there was no parallelism between the action of atropine and sectioning of the vagi nerves, and often the fall of blood pressure caused by oxygen persisted after atropinization but the effect of oxygen was converted into a rise of blood pressure by sectioning of the vagi nerves.

EFFECTS OF OXYGEN ADMINISTRATION ON HUMAN SUBJECTS DURING OXYGEN-WANT

In order to ascertain whether the two different reactions of the blood pressure to the administration of oxygen could be elicited in human beings as well as in experimental animals, various forms of experiments were tried on 30 volunteer medical students who had previously undergone numerous experimental ascents in the decompression chamber and whose tolerance to anoxia and individual responses to it were carefully studied and recorded. Some of the tested subjects had as many as 50 ascents in the decompression chamber, totalling 81 hr. The average time spent in the chamber by each of the 10 students used in the final series of experiments equalled 32 hr.

Two forms of experiments were developed that yielded most satisfactory results. These experiments were performed on corresponding series of students, many of whom participated in both experiments. The findings for these experiments are represented in diagrammatic form in Figs. 7 and 8.

The experiments consisted in a sudden interruption and resumption of the oxygen supply at altitudes ranging between 25,000 and 29,000 ft., and a gradual ascent to altitudes ranging between 16,500 and 20,000 ft. without oxygen, followed by administration of pure oxygen. The difference in the altitude and some difference in the duration of exposure to it depended on the individual resistance to anoxia of the tested subjects.

With the trained personnel at our disposal in which an emotional response did not complicate the reaction, these two forms of experiments made it possible to show that both reactions to oxygen administration that were described in animal experiments could be reproduced in human subjects.

Acute anoxia induced by means of removing the Boothby-Lovelace-Bulbulian oxygen mask (5) at an altitude ranging between 25,000 and 29,000 ft. caused, within a period of 30 to 40 sec., distinct facial pallor, an increase in the rate of the pulse, and a progressive rise of the systolic blood pressure. The diastolic pressure varied greatly in different experiments and only occasionally showed a distinct rise, which was followed in the later stages of anoxia by a decline. In three and one-half to six minutes (the time and altitude, as stated before, depending on the individual's resistance to anoxia), the systolic blood pressure became almost stable or began to show a slight tendency to decline, on the average reaching a level of about 20 to 25 mm. Hg higher than that prevailing before removal of the oxygen mask. The diastolic pressure during this period was usually below the level prevailing at the beginning of the experiment and, as seen in Fig. 7, was declining quite rapidly just before the introduction of oxygen. The pulse rate increased from 30

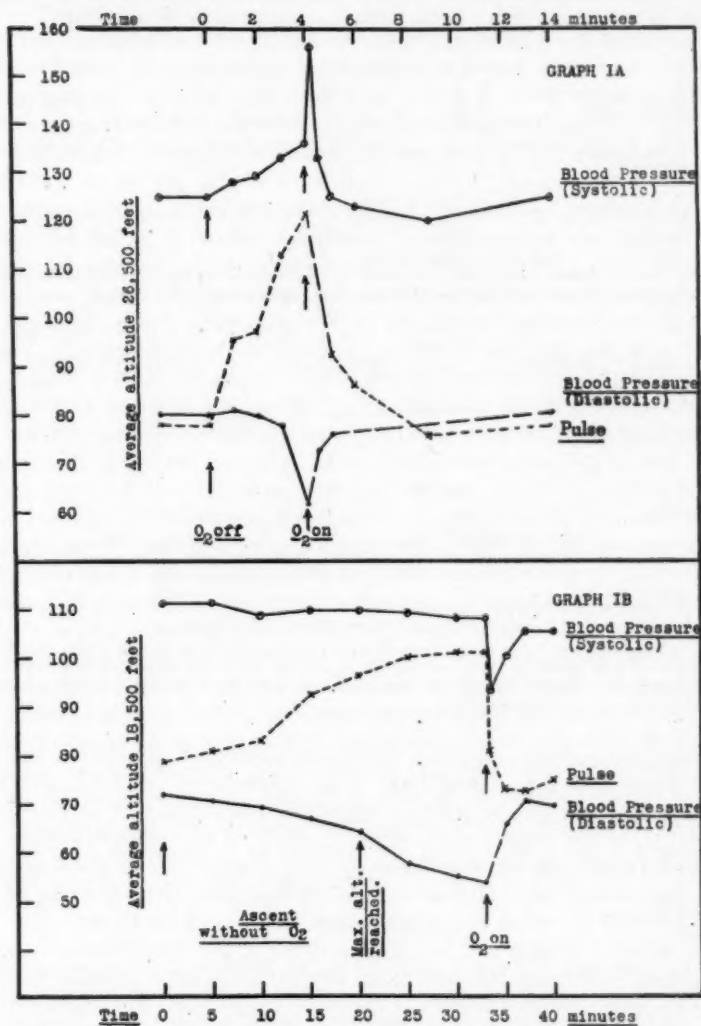


FIG. 7. A composite diagram, 10 ascents being represented in each graph.

Graph I A shows the effects on blood pressure and pulse of rapidly induced anoxia followed by oxygen. (Ascent with oxygen at a rate of 4000 ft. per minute to average altitude of 26,500 ft. Average time at altitude without oxygen equals 4.1 min. after which oxygen is administered at the rate of 1.5 to 3.0 litres per minute from a Boothby-Lovelace-Bulbulian mask).

Graph I B shows the effects on blood pressure and pulse of gradually induced anoxia followed by oxygen. (Ascent without oxygen at a rate under 1000 ft. per minute to average altitude of 18,500 ft. Average time at altitude without oxygen equals 13.1 min. after which oxygen is administered from a Douglas bag).

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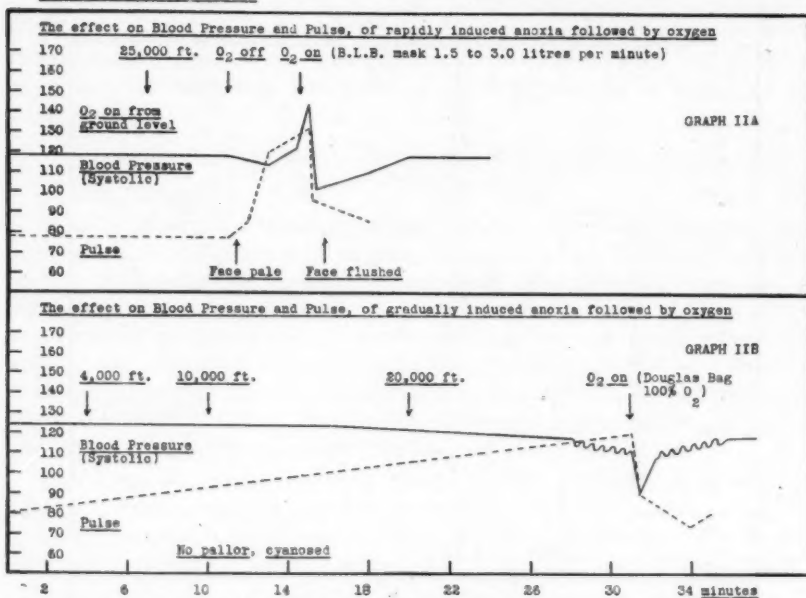


FIG. 8. Two individual ascents showing the effects on blood pressure and pulse of rapidly induced anoxia and of gradually induced anoxia followed by oxygen.

The different reactions to oxygen administration that take place in the two forms of experiments referred to in legend to Fig. 7 are shown in Graphs IIA and IIB to occur in one and the same subject. Note in Graph IIB the variations in blood pressure, which resemble Traube-Hering-Mayer waves.

to 55 beats per minute, cyanosis became marked, and the tested subjects became sleepy and dull, complained of the dimness of the light, and gave irregular gasps between the ordinary respiratory movements. At this point, reapplication of the oxygen mask, through which $1\frac{1}{2}$ to 3 litres of oxygen per minute were administered, usually resulted in a distinct rise of blood pressure ranging from 6 to 21 mm. Hg and was accompanied by a strengthening of the pulse. The rise of blood pressure occurred within 10 to 15 sec. after the administration of oxygen and was of a very short duration, lasting approximately 15 sec. This was followed by an abrupt fall of blood pressure, flushing of the face, and a marked slowing of the pulse (Figs. 7 and 8, Graphs IA and IIA). The tested subjects were asked to remember the values of the blood pressure readings, which were made aloud every 15 sec. and written down by an assistant. In most cases they could remember all the readings during the period of anoxia, but missed two or three during the drop of blood pressure, not realizing later that they had missed these readings. If the period of anoxia was not long enough or the amount of oxygen was increased, and instead of small quantities supplied by the flow metre, the tested subjects were given pure oxygen to breathe out of a Douglas bag (7), the decline of

blood pressure occurred so quickly that often no postanoxic rise of the same could be determined.

When, instead of a short period of anoxia, a gradual anoxia was induced by means of slow decompression that equalled an ascent at the rate of 1000 ft. per minute to the height of 16,500 to 20,000 ft. without any addition of oxygen, and then after a period of 6 to 20 min. at that altitude, oxygen was administered out of a Douglas bag (7), the reaction was of a quite different nature.

During the period of progressive anoxia (Figs. 7 and 8, Graphs I B and II B), the tested subjects became gradually flushed, and as the lack of oxygen developed, cyanosis was very pronounced owing to the concurrent engorgement of the cutaneous blood vessels. The systolic blood pressure usually showed no tendency to rise, and mostly a drop of blood pressure ensued, the diastolic pressure falling off more quickly than the systolic one, and the pulse becoming very rapid. In 8 out of 10 tested subjects included in the series shown in Fig. 7 (Graph I B), the decline of the systolic blood pressure during the period of anoxia amounted to 2 to 17 mm. Hg; in one it remained unchanged, and in one it rose 6 mm. Hg. The diastolic pressure invariably declined, falling from 12 mm. Hg to as much as 30 mm. Hg. The increase in pulse rate was from 16 to 36 beats per minute. A sudden administration of oxygen in these conditions resulted in a rapid and further fall of the systolic blood pressure, which was accompanied by an abrupt slowing of the pulse. The drop of the systolic blood pressure became prominent within 15 to 30 sec. after the beginning of the administration of oxygen; it amounted to 7 to 26 mm. Hg altogether averaging 14%, but on some occasions as much as 22% of the total blood pressure. This depression of the blood pressure lasted at times as long as one or two minutes, gradually returning to its previous level though often a much quicker restoration of normal conditions took place. The decline in pulse rate though abrupt, reached its maximum later than the fall of blood pressure, the pulse being slowest, in many instances two to five minutes after the beginning of the administration of oxygen. The reduction in the pulse averaged 12 to 46 beats per minute equalling in one subject as much as 48% of the rate during anoxia.

Discussion

Referring to the variability of the effects produced by anoxia under different experimental conditions, Mathison (18) suggested that anoxia caused both excitation and depression of the vasomotor centres and that the two phenomena were balanced one against the other, either depression or excitation predominating in specific instances. Basically, this conception still holds true, though the mechanism of the action of anoxia was found to be extremely complicated, the chemoreceptors playing a prominent part in the excitation of the vasomotor system, whereas the depression of circulation caused by oxygen-lack has been supposed to be due in part to the action of oxygen-lack on the nerve centres, and partly to a direct effect on the heart (8, 9, 12, 23).

As shown in the present investigation, the administration of oxygen after periods of asphyxia or anoxia may lead either to pressor or depressor effects, and the conditions under which these different effects are produced are not unlike the ones that determine the effects of oxygen-lack. Thus, suddenly induced asphyxia or inhalation of nitrogen leads to a rise in blood pressure as does the administration of oxygen after periods of acute asphyxia or anoxia, whereas gradually induced asphyxia or anoxia leads to a decline of blood pressure, oxygen causing a further fall in blood pressure when given in these conditions.

From the experiments performed on spinal preparations it seems legitimate to conclude that the rise of blood pressure that occurs on resumption of respiration or administration of oxygen, after periods of acute asphyxia or anoxia, is predominantly due to an abrupt revival and activation by oxygen of the sympathetic centres, which leads to an augmentation of the action of the heart and to an intensification of the vascular tone, the two phenomena contributing to the rise of blood pressure in a varying degree under different experimental conditions. However, it is likely that in intact anaesthetized animals the stimulating effect of oxygen is accomplished largely by way of the chemoreceptors, as sectioning of the vagi nerves and denervation and exclusion of the carotid sinuses abolishes it when oxygen is given following inhalation of nitrogen, under these conditions oxygen causing only a recovery of the pre-existing blood pressure level that was depressed by nitrogen.

It is also worth noting that in intact anaesthetized animals, the stimulating effect of oxygen is exerted directly through the sympathetic nervous system, the same being true in the case of stimulation by anoxia (14), while in spinal preparations placed on "minimal ventilation," the rise of blood pressure produced by oxygen depends largely on the presence of the suprarenal glands.

One more point of interest should be mentioned in connection with the study of the pressor action of oxygen. Gellhorn and Lambert (8) emphasize the importance of controlling the respiration in investigations of the effects of anoxia and asphyxia on the vasomotor system in animals with an intact central nervous system. The present experiments in spinal preparations show that the extent of artificial respiration may in itself profoundly alter the cardiovascular responses induced by periods of acute asphyxia. Thus, under conditions of "optimal" ventilation, asphyxia leads to a rise in blood pressure, whereas a previous period of "minimal" ventilation reverses this effect. In spite of this reversal of the effect of asphyxia, the effect of a subsequent administration of oxygen or of resumption of aeration remains unaltered, causing a marked rise of blood pressure.

Turning to the depressor effects of oxygen it must be pointed out that, as shown by Marshall and Rosenfeld (16), administration of oxygen did not affect the blood pressure level to any extent in spontaneously breathing animals. However, in the present investigation it was found that a previous inhalation of mixtures of gases deficient in oxygen reproduced, in experimental

animals, conditions prevailing in human beings breathing rarified air, in whom Schwarz and Malikiosis (28) and Schwarz (27) found that inhalation of 100% oxygen caused marked depression of the blood pressure and slowing of the heart.

Several possible mechanisms of this effect of oxygen are suggested. Among them Schwarz and Malikiosis (28) and Schwarz (27) mention a direct depressing effect of oxygen on the central nervous system and a sudden constriction of the cerebral blood vessels induced by the inhalation of oxygen in acapnic states caused by previous over-breathing, while Schmidt and Comroe (24) favour the possibility of a sudden release of the overactive chemoreceptors, this enabling the depression of the nerve centres caused by a previous hypo-oxygenation to come into its own.

The fact that no depression of the blood pressure by oxygen could be produced after sectioning of the vagi nerves and exclusion of the carotid sinuses, as well as in the spinal preparations, has indicated that the mechanism suggested by Schmidt and Comroe (24) must play a very important part in the depression of the circulation caused by oxygen. In this respect the analysis of the effect of carbon dioxide on the depressor action of oxygen has been quite interesting. As observed by Schwarz (27), when carbon dioxide was added to the oxygen, it did not counteract the depressor effect of oxygen, and in many instances seemed to enhance it. Further, it was shown by Marshall and Rosenfeld (16) that when the respiratory centre was depressed by anaesthetics, and the respiration was perpetuated largely by the chemoreceptors, oxygen caused a marked depression of respiration that was not affected by the addition of carbon dioxide, while Watt, Dumke, and Comroe (33) demonstrated that a reduction of ventilation occurred also in unanaesthetized dogs on oxygen administration, but that this effect of oxygen was increased by anaesthesia.

In the present investigation it was found that in the cat under light chloralose and urethane anaesthesia, the depression of breathing caused by oxygen was insignificant when the animal was previously breathing ordinary air, but was greatly exaggerated by preceding periods of hypo-oxygenation. In these conditions, complete cessation of respiration often occurred for 15 to 50 sec. after oxygen was given, and was followed by a gradual return to the normal respiratory rate and volume. This observation is in agreement with the view that periods of hypo-oxygenation depress the central nervous system, thus increasing the importance of the chemoreceptors in the regulation of the respiration and circulation. However, in the experiment presented in Fig. 5 C, addition of 5% carbon dioxide to the 9% oxygen that the animal breathed for 15 min. preceding the administration of 100% oxygen produced an increased irritability of the respiratory centre, which lasted for some time after the removal of the carbon dioxide. Under these conditions oxygen caused no apnoea, only a very gradual reduction of the respiration to its normal level taking place; in spite of this, oxygen still produced the usual fall of blood pressure. On the other hand, atropine occasionally reduced the fall of

blood pressure caused by oxygen but did not diminish the depression of respiration*. These observations suggest that there is no complete parallelism between the degree of the preceding depression of the nervous system and the decline of blood pressure that takes place on the administration of oxygen. Also they show that there is not always a close relation between changes in respiration and the depressor effects of oxygen administration on the circulation.

From all these experiments the impression was gained that in addition to the phenomenon suggested by Schmidt and Comroe (24), an activation by oxygen of the vagal and vasodilator centres, and possibly an overaction of the depressor reflexes mediated by the carotid sinuses and the nerves of Cyon during their recovery from anoxia, were partly responsible for the depression of blood pressure and slowing of the heart that occurred during the administration of oxygen. That an overaction of the sympathetic centres may take place during the administration of oxygen after a period of anoxia was demonstrated in the first part of this investigation, while a decreased effectiveness of the depressor reflexes mediated by the carotid sinuses was shown by Gellhorn and Lambert (8, p. 27) to occur during the inhalation of a 9% oxygen mixture. Such a multiple nature of the response would account both for the constancy with which the depressor effect of oxygen on the circulatory system was seen to occur in the given set of experimental conditions and for the variability of the action of atropine and of the sectioning of the vagi nerves inasmuch as the degree of participation in the response of the different mechanisms may vary in individual animals.

Both the pressor and depressor reactions to the administration of oxygen could be reproduced in unanaesthetized human beings. The rise of blood pressure caused by oxygen after periods of sudden anoxia was more difficult to elicit and was more transient than that seen in experimental animals. However, the form of experiment presented above, though yielding most consistent results, was by no means the only procedure in which administration of oxygen resulted in a rise of blood pressure. Thus as pointed out by Besserer (4), a return to higher barometric pressures in the decompression chamber was seen to cause a postanoxic rise of blood pressure.

In the instance of prolonged anoxia caused by gradual ascent in the decompression chamber, it was found that the blood pressure remained constant only up to a certain altitude, rising quite sharply if it was surpassed or if the ascent was too rapid. This critical level in our group of tested subjects was between 16,500 and 20,000 ft. and its approach was usually indicated by variations of the blood pressure that somewhat resembled Traube-Hering-Mayer waves (1). These variations in blood pressure appeared at regular intervals, recurring at a rate of three or four a minute, and reached as much as 10 mm. Hg. Out of the 10 persons subjected to the gradual ascent in

* Though special recordings of the respiratory movements have not been presented, the changes in respiration can be followed to a certain extent on the respiratory oscillations of the blood pressure in Figs. 5 and 6.

the experiment shown in Fig. 7 (Graph I B) the variations were present in seven individuals. The administration of oxygen in this stage of anoxia caused, in all but one tested person, the described decline of blood pressure.

It is interesting that Schneider (25, pp. 7-97), Armstrong and Heim (2), and more recently Goldie (11) observed that the reaction to anoxia may vary with changes in the rate of ascent. Armstrong and Heim (2) found that sudden ascents to 30,000 ft. resulted in loss of consciousness without fainting, whereas gradual ascents led either to fainting or loss of consciousness. They concluded that rapid ascents had a greater effect on the central nervous system but the slower ones exerted a greater strain on the cardiovascular system. On the other hand Goldie (11) noted that sudden deprivation of oxygen at altitudes above 25,000 ft. caused early loss of consciousness, whereas gradually induced anoxia led to impairment in the performance of mental tasks and in judgment. It should be pointed out that the different reactions of the circulatory system to anoxia can play an important part in these effects of oxygen-lack though it must be emphasized that in untrained individuals the vascular response is extremely variable, probably an emotional response complicating the action of anoxia.

The impression was gained throughout the investigation on human subjects that an abrupt deprivation of oxygen, in which vasoconstriction and anoxaemia were combined, was tolerated badly by some individuals, whereas a gradual deprivation of oxygen in which a vasodilator response predominated was poorly withstood by others. Also in subjects who were comparatively comfortable during the anoxia, there was occasionally seen to occur during the first minute of the administration of oxygen, dizziness, feeling of faintness, or a transient loss of consciousness, and in one subject a real convulsive seizure developed. All these manifestations coincided with the abrupt circulatory readjustments that developed during the sudden inhalation of oxygen.

Acknowledgments

Appreciation is expressed for the splendid co-operation on the part of the students of the University of Western Ontario Medical School who volunteered to act as test subjects for part of the work. The writer would like to thank also the London Association for War Research whose co-operation greatly facilitated this investigation.

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THE EFFECT OF INORGANIC AND ORGANIC IODIDES UPON THE OUTPUT OF RESPIRATORY TRACT FLUID¹

BY ELDON M. BOYD², M. C. BLANCHAE³, JOAN COPELAND³,
SHIRLEY JACKSON³, K. PHIN³, AND MARY STEVENS³

Abstract

In an investigation of the reputed expectorant action of iodides, some 300 to 400 rabbits and cats were urethanized and arranged for the collection of respiratory tract fluid (*R.T.F.*). Potassium iodide and the synthetic, organic iodides Siomine (N.N.R.), and Iod-Ethamine, given by stomach tube, significantly augmented the output of *R.T.F.* in doses comparable to the recommended human dose. Iodized proteins, such as Iodalbin (N.N.R.) and Iodo-Casein (N.N.R.), and iodized fatty acids or oils, such as Lipoiodine (N.N.R.), Iodostarine (N.N.R.), Lipiodol (N.N.R.), Oridine (N.N.R.), Stearodine (N.N.R.), Sajodin (N.N.R.), and Iodicin, had no effect upon the volume output of *R.T.F.*, even when given in a wide range of doses. The mechanism of expectorant action was studied with potassium iodide, which, when given to urethanized cats in which the anterior and posterior gastric branches of the vagus nerve had been severed, caused no increase in the output of *R.T.F.*, proving that the expectorant action was by way of a reflex from the stomach, probably up the afferent vagus to the medulla oblongata and then down the efferent vagus to the bronchial glands. No evidence was found that the expectorant action of iodides was due to a direct effect upon the bronchial glands, but after giving potassium iodide and Iod-Ethamine to cats and rabbits, there followed a marked increase in the concentration of iodine-containing compounds in the *R.T.F.*, and this latter may or may not account for the reputed mucus-liquefying effect of iodides.

For many years, iodides have been prescribed in expectorant mixtures for the relief of cough, especially cough associated with the dry type of chronic bronchitis, with bronchial asthma and bronchospasm, with bronchiectasis and emphysema. Since an expectorant drug is considered to increase the formation of secretions along the respiratory tract, or to augment their excretion, it was decided to investigate the action, if any, of various iodides upon the output of respiratory tract fluid (*R.T.F.*) in animals. This was done by a method elaborated in this laboratory by Perry and Boyd (15) and improved by Boyd, Jackson, and Ronan (5). By this technique, animals are anaesthetized with urethane, given parenterally, at an average dose of 1 gm. per kgm. body weight, a dose that produces about 20 to 30% surgical anaesthesia and takes the animal to the lower level of Guedel's plane I of surgical anaesthesia, a degree of narcosis that, we have found, slightly dulls but does not eliminate visceral reflexes from the stomach (6). *R.T.F.* is then collected through a T-tube ligated into the trachea, the straight arm leading to a graduated receiving tube and the side arm to an apparatus that conditions the inhaled air to body temperature and close to 100% relative humidity, all of the exposed connections being insulated to prevent condensation of moisture.

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Contribution from the Department of Pharmacology, Queen's University, Kingston, Ont.

² Professor of Pharmacology.

³ Research Assistant in Pharmacology.

Experimental studies upon the expectorant action of iodides have been entirely confined to inorganic iodides, usually potassium iodide, have been relatively few in number, and have appeared irregularly over the last 50 or 60 years. The first work that we have encountered was reported in 1884 by Prosser James of the London Hospital Medical School (13) who, using the laryngoscope, found an increase in the output of mucous secretions in the respiratory tract following presentation of iodides by mouth. In 1896, James Calvert wrote that he had exposed the tracheal mucosa of urethanized cats and that, following the technique of Rossbach (1882), he had dried the mucosa with blotting paper, noted the interval before moisture could be seen again over the dried area, and found that iodides shortened this interval and augmented the amount of visible secretions (7). In 1910, Professor V. E. Henderson and A. H. Taylor of the University of Toronto described a method whereby they collected moisture and fluids from the respiratory tract in calcium chloride tubes; when potassium iodide was injected intravenously, there followed no change in the rate of increase in weight of calcium chloride tube; from this Henderson and Taylor concluded that if iodides augment the output of bronchial fluids, it must be through a reflex mechanism from the stomach (12). This reflex nature of the expectorant action of potassium iodide, which we have confirmed by direct experiment, has been lost sight of, or ignored, since the publication of the important work of Henderson and Taylor; in most textbooks and reviews, for example the review of Professor J. A. Gunn in 1927 (11), authors have reverted to the old view that iodides act directly by irritating the mucosa of the respiratory tract, an idea that we have found to be entirely incorrect.

More recently, Dr. T. Gordonoff and his associates of the University of Berne in Switzerland have studied the action of expectorant drugs chiefly by noting changes in the X-ray shadows of the chest after placing lipiodol in the bronchi of man. If an expectorant drug made the contrast media diffuse and pale to X-rays, Gordonoff concluded that the drug had increased the output of secretions that diluted the lipiodol and he referred to such expectorants as secretolytic. If this action did not occur but the lipiodol shadow was actively moving about and expectorant effects were obtained, Gordonoff categorized the drug as a secretomotor expectorant. In 1931, Gordonoff and Merz (10) reported that potassium iodide given per ora acted as a secretolytic expectorant. In 1934 (8), noting that toxic doses of potassium iodide or iodine produced pulmonary oedema, Gordonoff concluded that iodides act by liberation of free iodine in the lungs—"Beim Jodkalium handelt es nicht um eine Salzwirkung, sondern um eine reine Jodwirkung." Gordonoff's postulated mechanism of action of iodides was not only unsubstantiated by experiment, but was also based upon reasoning that is obviously open to severe criticism. In 1936, Gordonoff and Lehman (9) advanced the idea that iodides act as expectorants by impairing the reabsorption, in the lungs, of pulmonary secretions, because they found that postabsorptive blood

sugar curves, following intrabronchial administration of sugar solution, were delayed if potassium iodide had been given beforehand.

Stanley Alstead of Glasgow developed another technique. Using patients with chronic, exudative bronchitis, he measured the volume output of sputum before and after giving various expectorant drugs, which included potassium iodide (1) and none of which were found to increase the volume of sputum. Dr. Alstead reached the conclusion that the commonly used expectorant drugs were not expectorant at all. The fundamental error in Alstead's deduction is that he has confused expectoration, or spitting, with expectorant action, a mistake that is made rather commonly and that has been discussed in a previous communication from this laboratory by Perry and Boyd (15). Finally, it may be noted that Basch *et al.* reported in 1941 (3) that material collected by bronchoscopic drainage of the lungs of children with bronchiectasis had a lower pH and viscosity after the administration of iodides.

Potassium Iodide and the Output of *R.T.F.*

Potassium iodide is the iodide most commonly prescribed in expectorant mixtures and it was decided to begin with this salt and measure its effect, if any, upon the volume output of *R.T.F.* in rabbits and cats, as determined by the technique outlined above (5, 15). The normal output of *R.T.F.* was measured over a period of three hours (and expressed as ml. per kgm. body weight per 24 hr.), the drug was then given in various doses by stomach tube, and further hourly readings taken for a period of five or six hours. Controls were given water alone, in place of the solution of potassium iodide. Preliminary experiments revealed that potassium iodide did augment the output of *R.T.F.* and that the minimal effective dose was of the order of 0.1 gm. per kgm. body weight. Using doses of from 0.1 to 5 gm. per kgm. body weight, experiments were performed upon some 50 cats and 50 rabbits. The results were tabulated, averaged, and, in order to present them in a uniform manner, any change effected by the drug was calculated as a percentage of the average output of *R.T.F.* over the period of two hours immediately preceding administration of potassium iodide or water. These percentage changes were then plotted against time and typical results have been selected and presented in Fig. 1.

There was no appreciable change in the output of *R.T.F.* in either cats or rabbits given water alone. Potassium iodide in a dose of 0.1 gm. per kgm. body weight increased immediately the output of *R.T.F.* some 25 to 50% and the augmented output was maintained, at slightly lower levels, for a period of some six hours. When the dose of potassium iodide was increased to 0.5 or 1.0 gm. per kgm. body weight, there was a sudden and marked rise in the output of *R.T.F.* and, while it fell off slightly, the increased output was maintained for several hours. The largest dose of 5 gm. per kgm. caused a very marked rise in the volume output of *R.T.F.*

These results leave no doubt that potassium iodide increases the output of *R.T.F.* in rabbits and cats and if the results in these species may be extra-

polated to man, it may be concluded that potassium iodide has been proved to be an expectorant drug, in the sense that it augments the output of *R.T.F.* While the doses used were larger than those commonly recommended for human therapy, it should be recalled that these animals were anaesthetized,

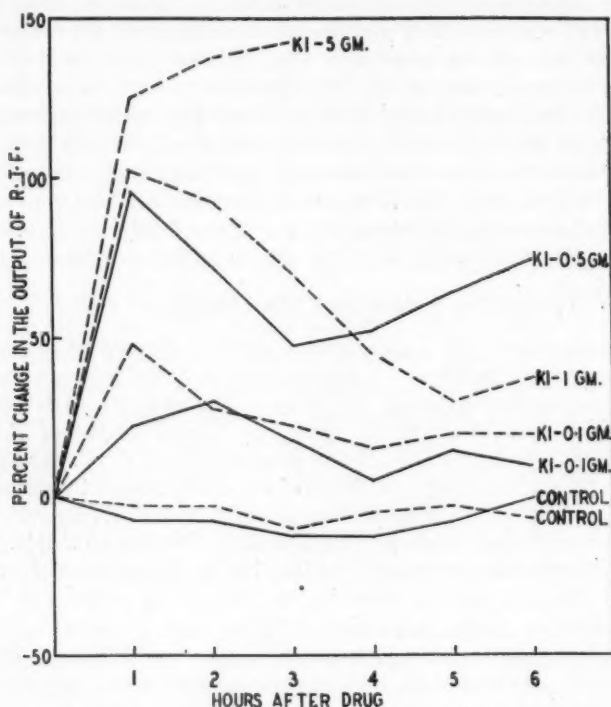


FIG. 1. The effect of potassium iodide upon the volume output of *R.T.F.* in cats (solid lines) and rabbits (interrupted lines).

that potassium iodide acts by way of a reflex, as will be shown below, and that anaesthesia depresses this reflex action to some extent so that larger doses are required than in the unanaesthetized animal. It has been proved in this laboratory, in unpublished experiments, that the dose of an expectorant drug (*Ipecacuanha* was used) that will produce a definite increase in the volume output of *R.T.F.* is much smaller in decerebrate cats under no anaesthesia than in anaesthetized cats.

Iodized Proteins, Fats, Fatty Acids, and Oils

Iodized proteins, fats, fatty acids, and oils were introduced into the therapeutic armamentarium some years ago with two claims (*a*) that they were less irritant than inorganic iodides upon the stomach—a claim that has

been substantiated by subsequent work—and (b) that they were less liable to cause the toxic reaction of iodism—a claim that has not been borne out by subsequent work because, dose of iodine for dose of iodine, they are just as liable to produce iodism as inorganic iodides (2). A number of these compounds may be found listed in *New and nonofficial remedies* (2) and most of them have been investigated as to their effect upon the output of *R.T.F.* One compound, Lipoiodine (N.N.R.), (the ethyl ester of diiodobrassicic acid, generously supplied by the Ciba Company Limited of Montreal) was selected for detailed study as a typical example of this group of drugs. The remaining compounds were studied in a more cursory manner.

TABLE I

THE HOURLY PERCENTAGE CHANGE IN THE OUTPUT OF RESPIRATORY TRACT FLUID FOLLOWING THE ORAL ADMINISTRATION OF LIPOIODINE

Species	Dose (mgm./kgm.)	No. of animals	Change in output of <i>R.T.F.</i> , %					
			Hour					
			1st	2nd	3rd	4th	5th	6th
Rabbit	0	16	0	- 8	0	8	23	20
Rabbit	10	14	22	22	- 6	0	-22	6
Rabbit	100	9	6	0	6	6	20	23
Rabbit	500	5	-10	-30	-20	-10	-10	-20
Rabbit	1000	7	0	6	0	12	-12	12
Cat	0	15	0	-24	- 8	-12	-25	- 4
Cat	100	7	-15	-15	-23	-23	- 8	0
Cat	1000	7	0	18	12	12	12	-23

Lipoiodine was given by stomach tube, in doses ranging from 0 to 1000 mgm. per kgm. body weight, to 51 rabbits, and, in a more circumscribed range of doses, to 14 cats, in a manner similar to that of potassium iodide. The results in the various dosage groups were averaged and any change in the mean expressed as a percentage of the average output of *R.T.F.* during the two hour period just before the drug was given. These data have been summarized in Table I. It is obvious that Lipoiodine (N.N.R.) did not appreciably or significantly affect the output of *R.T.F.* Again, if these results may be extrapolated to man, the conclusion must be drawn that while Lipoiodine may be useful as a substitute for inorganic iodides in other indications for iodide therapy, it is not an expectorant, in the sense that it does not augment the output of *R.T.F.*

Similar experiments were performed upon rabbits, using iodized proteins and other iodized fats, fatty acids, and oils. The general procedure was to start with a dose corresponding to 1/5th to 1/10th the average human dose per kgm. body weight and, giving each dose to two or more rabbits, work up to doses several hundred times the recommended human dose per kgm. body weight. As before, the drugs were given by stomach tube three hours after

the animals had been arranged for collection of *R.T.F.*, and to summarize the data, the mean changes in the volume output of *R.T.F.* over a period of four hours after giving the drug were expressed as a percentage of the mean output over the two hours just before the drug was given. These data, obtained upon a total of 117 rabbits, have been collected in Table II.

TABLE II

THE MEAN PERCENTAGE OF VARIOUS IODIZED PROTEINS, FATS, FATTY ACIDS, AND OILS UPON THE VOLUME OUTPUT OF *R.T.F.* OVER A PERIOD OF FOUR HOURS AFTER ADMINISTRATION TO RABBITS

Iodide	Dose, mgm. per kgm.	No. of animals	Mean change over four hours, %
Iodo-Casein	5 to 2500	16	26
Iodalbin	30 to 15,000	12	7
Iodostarine	2 to 400	12	-23
Oridine	4 to 400	10	2
Stearodine	4 to 800	14	10
Sajodin	50 to 10,000	12	-29
Iodicin	3 to 600	12	-21
Lipiodol	50 to 500	12	10
Control	0	17	- 6

Two iodized proteins were studied, namely, Iodalbin (N.N.R.), which is an iodized blood albumin containing about 22% of iodine and kindly provided by Parke, Davis and Company, and Iodo-Casein (N.N.R.), which is an iodized milk casein containing some 18% of iodine and kindly provided by Sharp and Dohme, Inc. The following iodized fats, fatty acids, or oils were studied: Iodostarine (N.N.R.), which is diiodotaric acid containing some 48% of iodine and kindly provided by Hoffmann-LaRoche, Inc.; Lipiodol (N.N.R.), which is iodized poppy-seed oil containing some 40% of iodine and kindly provided by Vinant Limited; Lipoiodine (N.N.R.), which has already been described; Oridine (N.N.R.), which is the calcium salt of iodized cottonseed oil containing some 24% of iodine and kindly provided by Eli Lilly and Co.; Stearodine (N.N.R.), which is the calcium salt of iodostearic acid containing some 27% of iodine and kindly provided by Parke, Davis and Co.; Sajodin (N.N.R.), which is calcium iodobenhenate containing some 24% of iodine and kindly provided by the Winthrop Chemical Company; and Iodicin, which is calcium iodicinoleate kindly provided by Burroughs Wellcome and Company.

It may be seen from Table II that none of these compounds had an appreciable effect upon the volume output of *R.T.F.* in rabbits. The fact that these compounds have little or no irritant effect upon the gastric mucosa suggests that the expectorant action of potassium iodide may have been due to a reflex from the stomach, an idea that will be dealt with below and one made in 1910 by Henderson and Taylor (12).

Organic Iodide Substitutes

Iodides and iodide substitutes for systemic use may be classified after the following manner into (a) inorganic iodides, (b) iodized proteins, (c) iodized fats, fatty acids, and oils, and (d) what we have termed organic iodide substitutes. We admit that the latter term is not entirely satisfactory, but it is meant to include synthetic organic iodides not included in Groups (b) and (c) and which may be used for the systemic effect of iodide. Two compounds in Category (d) were investigated, namely, Siomine (N.N.R.), which is hexamethylenetetramine tetraiodide containing some 79% of iodine, and Iod-Ethamine, which is ethylenediamine dihydriodide containing some 80% of iodine; both of these compounds were kindly supplied by the Pitman-Moore Company of Indianapolis. Since both Siomine and Iod-Ethamine augmented the output of *R.T.F.*, like potassium iodide of Category (a) but unlike the compounds in Categories (b) and (c), it was deemed advisable to include them in a separate classification.

Siomine was given by stomach tube to 14 rabbits in doses of from 20 mgm. per kgm. body weight upwards and since all doses augmented the output of *R.T.F.* to about the same extent, it may be concluded that the maximal effect was obtained with the 20 mgm. dose. Hence all of the data were averaged and plotted in Fig. 2. Iod-Ethamine was similarly given to 18 cats and 18 rabbits in doses of 10, 100, and 1000 mgm. per kgm. body weight. All doses produced an increase in the volume output of *R.T.F.* and two typical curves have been included in Fig. 2.

In summary, therefore, it may be stated that examples of iodides in Category (a)—potassium iodide—and in Category (d)—Siomine and Iod-Ethamine—but no examples of iodides in Categories (b) and (c), significantly increased the output of *R.T.F.* in the animals studied and under the conditions of the experiment.

The Mechanism of Action

Most evidence upon the mechanism of action of iodides, apart from their function in the physiology of the thyroid gland, has been based upon theoretical and armchair reasoning. Thus, in 1860, Wood (17) stated that iodine was an essential component of the body concerned with, "that function of the body by which all of its parts are undergoing constant disintegration and renewal," iodides tending to accelerate this process. Later, in 1874, Stillé (16) concluded that iodide, "tends to eliminate from the system, first, those constituents of the body which are most slightly incorporated with it, and which are, indeed, foreign to its structure, and its action ultimately becomes pathological instead of salutary, when it promotes the disintegration and discharge of the organic elements themselves." It is difficult for the modern pharmacologist to comprehend the thought processes that gave birth to such statements and quite impossible to understand what is meant by the statements themselves.

Liebreich and Issersohn (See 14) administered potassium iodide, found that first potassium was increased in amount in urine and then the iodide, and concluded that potassium iodide was broken down in the body to yield

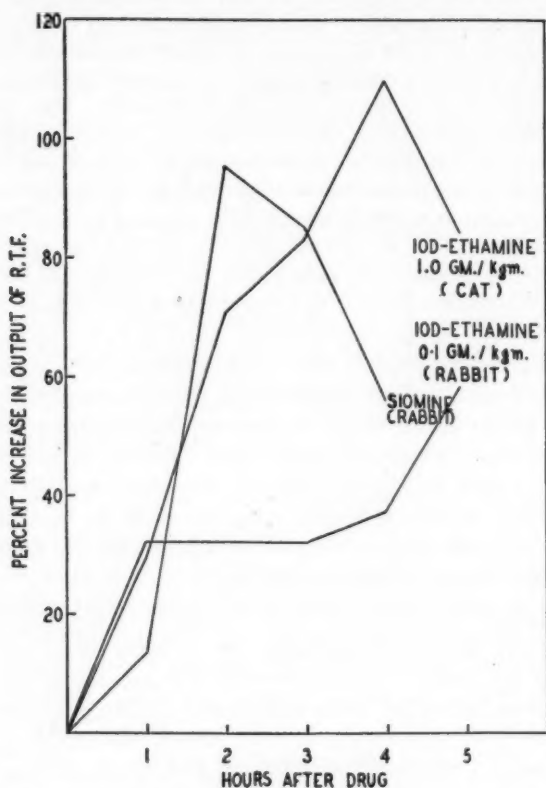


FIG. 2. The effect of Iod-Ethamine and Siomine upon the volume output of R.T.F. in cats and rabbits.

potassium ion and free iodine or iodide. Binz (See 14) showed that potassium iodide formed free iodine in the presence of protoplasm and carbonic acid. As a result of these pieces of evidence, Buckheim (See 14) arrived at the conclusion that potassium iodide in the body formed an iodine-albuminate in the walls of the blood vessels and this, in turn, led to increased permeability.

In 1910, Henderson and Taylor gave potassium iodide intravenously, found that, by their technique, it did not increase the output of bronchial fluids and concluded that it must act reflexly from the stomach and not, as previously supposed, directly upon the bronchial glands. The concept of a direct action of iodides upon the bronchial glands persisted, apparently supported by various bits of circumstantial evidence, and as late as 1940, Young (18)

stated, without giving any experimental evidence of his own, that the action was a direct one.

Our results are in harmony with the conclusions of Henderson and Taylor. Potassium iodide was given by stomach tube, in a dose of 1 gm. per kgm. body weight, to cats in which the afferent gastric nerves had been previously severed. This dose of potassium iodide has been previously shown to augment the output of *R.T.F.* in animals with the gastric nerves intact. When the afferent gastric nerves were severed, experiments in 16 cats demonstrated that in none was there any significant effect upon the output of *R.T.F.* volume. Thus by direct experimentation, we have been able to show that the expectorant action of potassium iodide is through a reflex via the vagus nerve and originating in the stomach.

The Output of Iodine in *R.T.F.*

The idea that iodides act directly upon the bronchial glands to augment the output of *R.T.F.* is essentially based upon the concept that an iodine-

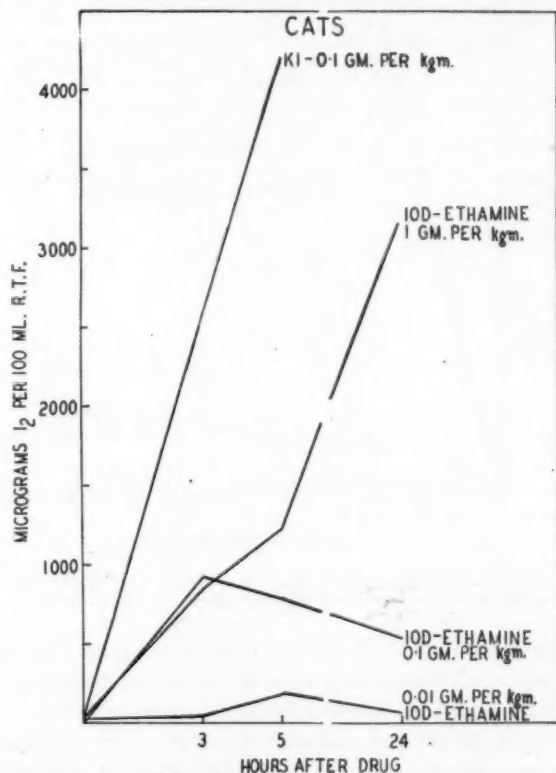


FIG. 3. The effect of potassium iodide and Iod-Ethamine upon the concentration of iodine-containing substances, estimated as free iodine, in the *R.T.F.* of cats.

containing substance can be demonstrated, after giving iodides by mouth, in large amounts in various cells, including the cells lining the respiratory tract. We have made direct experiments to find if iodine-containing substances appear in large amounts in *R.T.F.* after iodides have been given by mouth and have found this to be so. In these experiments, six or more rabbits or cats were each given stated doses of potassium iodide or one of the organic iodides—Iod-Ethamine, which augmented the output of *R.T.F.* At intervals up to 24 hr. after administration of the iodide, samples of *R.T.F.* were analysed for their iodine content by the method of Boyd and Clarke (4). In both rabbits and cats, there was a marked increase in the concentration of iodine-containing substances in *R.T.F.* under these conditions. The averaged results have been plotted in Figs. 3 and 4. The presence of these iodine-

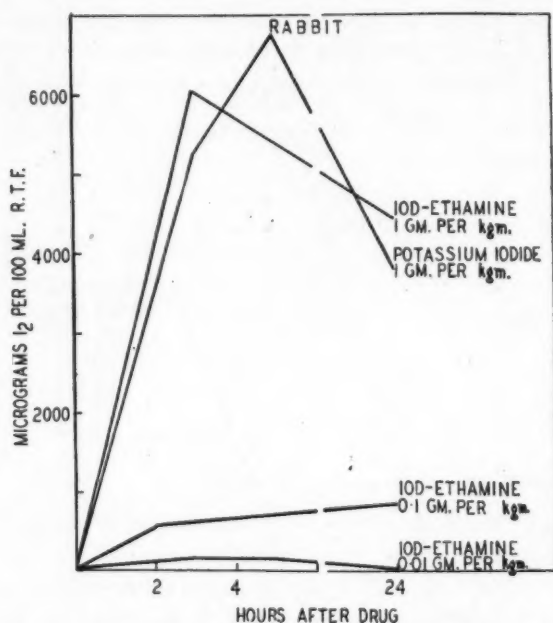


FIG. 4. The effect of potassium iodide and of Iod-Ethamine upon the concentration of iodine-containing substances, estimated as free iodine, in the *R.T.F.* of rabbits.

containing substances in *R.T.F.* did not, however, explain the expectorant action of iodides, which has already been shown to be through a reflex from the stomach. An excess of iodine-containing compounds in *R.T.F.* might explain the lowering of pH and viscosity of *R.T.F.* noted by Basch *et al.* (3) and hence account for the reputed beneficial effect of iodides in coughs associated with thick, tenacious mucus and in asthmatic conditions. On

the other hand, a simple increase in the output of normal *R.T.F.*, which has a low viscosity and a pH near the neutral point (15), could just as well account for the therapeutic effect of iodides in such conditions.

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THE EFFECT OF POTASSIUM IODIDE, SODIUM IODIDE, AND IOD-ETHAMINE UPON THE CONCENTRATION OF ALCOHOL-SOLUBLE AND ALCOHOL-INSOLUBLE FRACTIONS OF BLOOD IODINE¹

BY ELDON M. BOYD² AND M. C. BLANCHAE³

Abstract

Potassium iodide, sodium iodide, and Iod-Ethamine were given by stomach tube to 54 rabbits and the concentration of alcohol-soluble and -insoluble blood iodine followed for a period up to 24 hr. Following all three iodides, there was a rise and fall in the values of both fractions of blood iodine, the values for the alcohol-soluble fraction rising to the higher levels but declining more rapidly than the values for the alcohol-insoluble fraction. Potassium iodide had the advantage over sodium iodide that after its use the peak levels of both fractions of blood iodine were maintained for a longer period of time. Iod-Ethamine had the advantage over potassium iodide that higher peak levels were reached with both fractions of blood iodine and, while not maintained for as long as after potassium iodide, these peak levels were held for several hours and for a longer period than following sodium iodide. The concentrations of blood iodine were of the same order or higher than those found in respiratory tract fluid following administration of the same dose of iodides, suggesting that the appearance of iodine-containing substances in respiratory tract fluid (*R.T.F.*) after iodide therapy is of the nature of a simple diffusion from blood.

The purpose of the investigation to be reported below was threefold. In the first place, Boyd *et al.* (2) have shown that potassium iodide and Iod-Ethamine, given by stomach tube to rabbits and cats, increase the output of respiratory tract fluid (*R.T.F.*), and hence may be considered to have properties of expectorant drugs. Incidental to the expectorant action, but not causative of it, there was found to occur a marked increase in the concentration of iodine-containing substances in the *R.T.F.* We have found that normal *R.T.F.* of cats and rabbits has about the same concentration of measurable iodine as has blood, that is, from some 10 to 20 $\mu\text{gm. per } 100 \text{ ml.}$ ($\mu\text{gm. } \%$), and we have considered that iodine-containing substances simply diffuse into *R.T.F.* from blood. After giving potassium iodide or Iod-Ethamine by stomach tube in doses of 1 gm. per kgm. body weight, the concentration of measurable iodine rose to values of the order of 5000 $\mu\text{gm. per } 100 \text{ ml.}$ of *R.T.F.* These very high values suggested that iodine-containing compounds might be actually secreted into the *R.T.F.*, unless it could be shown that blood contained values for measurable iodine, under the above conditions, just as high as, or even higher than, was found in the *R.T.F.* Hence it was decided to give these iodides by stomach tube in doses of 1 gm. per kgm. body weight and follow the changes in the concentration of blood alcohol-soluble ("inorganic") iodine and alcohol-insoluble ("protein") iodine.

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Contribution from the Department of Pharmacology, Queen's University, Kingston, Ont.

² Professor of Pharmacology.

³ Research Assistant in Pharmacology.

In the second place, Dr. E. D. Osborne of the Mayo Clinic reported some years ago (4), that potassium iodide given to patients produced an increase in blood "protein" iodine while administration of sodium iodide had little effect upon the level of blood "protein" iodine. While Osborne's work did not prove that potassium iodide was preferable to sodium iodide therapeutically, it at least indicated that there was apparently a biochemical difference in the action of the two compounds and suggested a foundation in fact for the continued use of potassium iodide, rather than sodium iodide, in such pharmaceutical preparations as *Liquor Iodi Aquosus*, B.P. (1) and *Liquor Iodi Fortis*, U.S.P. (5), both of which are commonly called Lugol's Solution. Osborne also reported that sodium iodide is excreted in urine at a rate faster than that of potassium iodide which suggested that potassium iodide has the advantage of a more prolonged action than sodium iodide. Since we were investigating the effect of potassium iodide upon the two fractions of blood iodine, advantage was taken of this opportunity to include sodium iodide in order to ascertain whether we could confirm Osborne's findings.

In the third place, it was desired to find whether the organic iodide, Iod-Ethamine, has any advantage in the way of prolonged postabsorptive blood iodine curves, over the two inorganic iodides. Of the many organic iodides available, Boyd *et al.* (2) have found only two, namely Siomine (N.N.R.) and Iod-Ethamine that have expectorant properties like those of the inorganic iodides. Iod-Ethamine was selected as an example of an expectorant organic iodide. Chemically, it is ethylenediamine dihydriodide, containing about the same percentage of iodine as sodium iodide and potassium iodide, and it was kindly provided by the Pitman-Moore Company of Indianapolis, through the courtesy of Dr. Frank B. Fisk. By comparing Figs. 1 and 2 of the paper by Boyd *et al.* (2), it may be seen that Iod-Ethamine had a more prolonged, and, dose for dose, a more marked, expectorant effect than potassium iodide in the rabbit but little difference could be detected in the cat.

Postabsorptive blood iodine curves were determined in the following manner. Three groups of 18 rabbits each were given by stomach tube, 1 gm. per kgm. body weight of the following iodides: Group *a*, potassium iodide; Group *b*, sodium iodide, and Group *c*, Iod-Ethamine. Samples of venous blood were oxalated and taken at intervals of 0, 0.5, 1.5, 2.5, 3.5, 6, 9, 15, 18, and 24 hr. after administration of the iodides. In view of the high values found for blood measurable iodine, a sample of about 2 ml. of blood was found to be sufficient for analysis in all except the sample taken before the iodides were given, when 10 to 15 ml. were required. Blood was then extracted repeatedly with alcohol, which had been especially purified, after the method of Boyd and Clarke (3). It is our custom, in this laboratory, to refer to the iodine contained in this extract as the cold-alcohol-soluble fraction and it is analogous to what is usually referred to as the non-protein blood iodine. Similarly, we refer to the iodine content of the residue after extraction with alcohol at room temperature, as the cold-alcohol-insoluble fraction and this corresponds approximately to what is usually called protein blood iodine. The terms

protein and non-protein blood iodine are somewhat ambiguous in so far as they refer to alcohol-separated blood iodine, because some analysts have used alcohol at room temperature, some, alcohol in an extraction apparatus at 78° C., and the length of time and other factors have varied considerably. Boyd and Clarke (3) have shown that the conditions of the experiment must be clearly defined because, under certain conditions, all iodine in blood can be made soluble in hot alcohol. Using alcoholic separation, at least three fractions of blood iodine may be separated (3) but as we have no proof of the exact chemical nature of these fractions, we prefer to use the terms cold-alcohol-soluble and -insoluble in connection with the fractions herein separated, rather than the terms protein and non-protein to which, in past usage, these fractions roughly correspond. The iodine content of the two fractions was then determined with the aid of a Conway microburette after the method in use in this laboratory (3).

Values for the concentration of these two fractions of blood iodine, as determined before and at the stated intervals after the various iodides were given by stomach tube, were tabulated, averaged, and plotted in Figs. 1 and 2. The mean changes in the concentration of cold-alcohol-soluble iodine have been charted in Fig. 1. Rising from low initial pretreatment values that averaged slightly less than one-half the normal total blood iodine, the concentration rose rapidly within an hour or two to values between 12,000 and 24,000 $\mu\text{gm. per } 100 \text{ ml. of whole blood}$ and then fell off gradually until at 24 hr. after administration of the iodides the values were between 4000 and 12,000 $\mu\text{gm. \%}$. The following differences were noted between the post-absorptive curves after the three iodides. Following the administration of all three iodides, there was a rapid initial rise in the level of cold-alcohol-soluble iodine. The greatest initial rise followed administration of sodium iodide, which reached a peak value of nearly 24,000 $\mu\text{gm. \%}$ in about two hours, the peak value was maintained for only an hour or so and then the level fell rapidly until about nine hours after the drug was given, when the slope of the drop became less precipitous and declined gradually from about 11,000 $\mu\text{gm. \%}$ at nine hours to about 5000 $\mu\text{gm. \%}$ at 24 hr. Following administration of potassium iodide, the concentration of cold-alcohol-soluble blood iodine rose rapidly within half an hour to about 9000 $\mu\text{gm. \%}$, then more slowly increased to 15,000 to 16,000 $\mu\text{gm. \%}$ at six hours; this level or plateau was held for several hours to 15 to 18 hr. and then declined gradually to about 11,000 $\mu\text{gm. \%}$ at 24 hr. These results indicate that a high blood level of alcohol-soluble iodine is maintained for a longer period of time following the use of potassium iodide than following sodium iodide. The rapid decline in the concentration of cold-alcohol-soluble blood iodine following use of sodium iodide might be interpreted as indicating that the iodide has been changed into an alcohol-insoluble form, or that this fraction of blood iodine has gone into the tissues, or that it has been eliminated in urine. As will be shown below, the first of these possibilities does not occur and in view of the work of Osborne (4), it would appear most likely that a rapid renal excretion

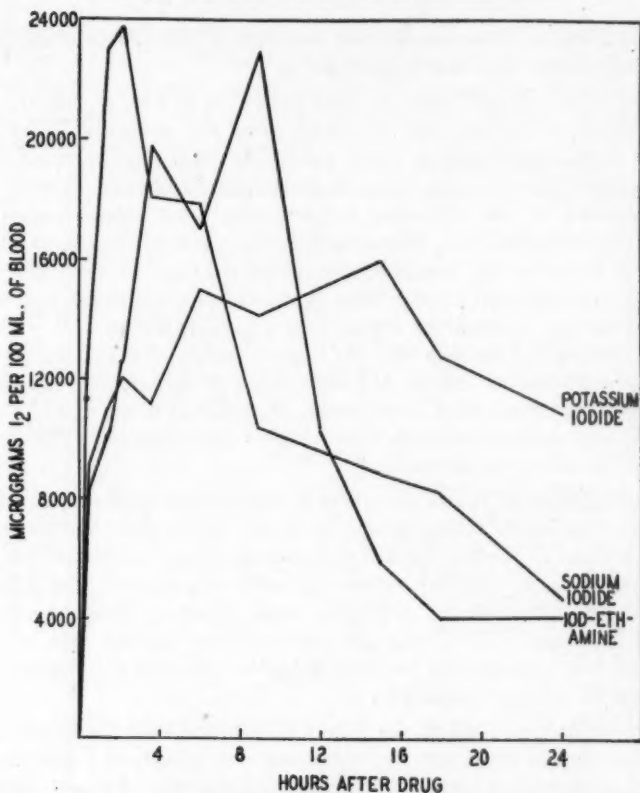


FIG. 1. The effect of administration by stomach tube of potassium iodide, sodium iodide, and Iod-Ethamine, in doses of 1 gm. per kgm. body weight, upon the concentration of cold-alcohol-soluble or "non-protein" blood iodine of rabbits.

of sodium iodide accounted for the rapid fall in the concentration of this fraction. The combination of these various factors would seem to justify, therefore, the continued use of potassium iodide, rather than sodium iodide, in instances where a therapeutic effect of iodide after absorption into the blood stream is desired, as in the preparation of patients for thyroidectomy.

There is good reason to believe that the cold-alcohol-soluble fraction of blood iodine consists mostly of inorganic iodide and water-soluble iodide. As the level of this fraction in blood was even greater than the level in *R.T.F.*, following the administration of equivalent doses of the iodides, it becomes unnecessary to postulate that iodide therapy results in a secretion of iodide into *R.T.F.* Simple diffusion from blood to *R.T.F.* could readily account for the high values found by Boyd *et al.* (2) in the *R.T.F.* The evidence herein obtained does not prove that iodine-containing compounds get into *R.T.F.*

by simple diffusion from blood; the evidence is only circumstantial but is strongly suggestive that this is the case.

Is there any evidence, from the data presented in Fig. 1, that the organic iodide Iod-Ethamine has any advantage over the inorganic iodides? The curve for cold-alcohol-soluble blood iodine obtained after administration of Iod-Ethamine differed from that obtained after administration of the inorganic iodides in the following respects: the peak concentration almost equalled that obtained after sodium iodide was given but was maintained not for an hour or so but for five or six hours and the concentration reached, the peak concentration, was greater than that following use of potassium iodide though it was not maintained for as long a period of time. It would seem logical to conclude from this that the organic iodide, Iod-Ethamine, has the advantage over sodium iodide that high levels of cold-alcohol-soluble blood iodine could be maintained with longer intervals between dosing, and the advantage over potassium iodide that a higher concentration of cold-alcohol-soluble blood iodine can be attained.

Average changes in the concentration of cold-alcohol-insoluble blood iodine, so-called protein iodine, following administration of the three iodides have been plotted in Fig. 2. Following all three iodides, the concentration of this fraction rose rapidly within one hour of administration of the drugs, to a level of about 1000 $\mu\text{gm. \%}$, and then more slowly rose to values between 3000 and 5000 $\mu\text{gm. \%}$. The peak concentration reached was only about one-quarter that attained by the cold-alcohol-soluble blood iodine but it was maintained for a longer period of time.

We were unable to confirm the report of Osborne that the administration of potassium iodide does, but the administration of sodium iodide does not, cause an increase in the concentration of this fraction of blood iodine. In fact, the average concentration of the alcohol-insoluble fraction was increased to a greater extent following sodium iodide than following potassium iodide. On the other hand, following sodium iodide the concentration of this fraction fell more rapidly from its peak value than it did following administration of potassium iodide, which could be harmonized with Osborne's finding that sodium iodide is more readily excreted in urine than potassium iodide.

The postabsorptive curves for cold-alcohol-insoluble iodine reached about the same levels as those found in *R.T.F.* following administration of the same dose of the iodides. It is possible that some, at least, of this fraction of blood iodine could diffuse into the *R.T.F.* If such is so, then the evidence obtained and plotted in Fig. 2 adds further weight to the suggestion that the occurrence of iodine-containing substances in *R.T.F.* is by way of simple diffusion.

Finally, a comparison of the different iodides leads to the conclusion that Iod-Ethamine has the same advantages over sodium iodide and potassium iodide with respect to its effect upon the concentration of the cold-alcohol-insoluble blood iodine as it had upon the concentration of cold-alcohol-

soluble iodine, though not to quite as marked a degree. The peak plateau reached following Iod-Ethamine was almost as great as that following sodium iodide but maintained for a longer period of time and the peak plateau was somewhat greater than that following potassium iodide but not maintained for quite as long.

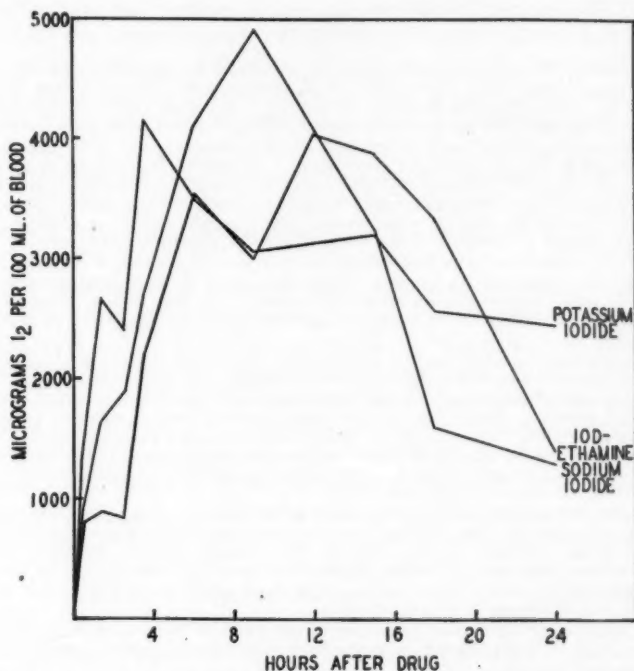


FIG. 2. The effect of administration by stomach tube of potassium iodide, sodium iodide, and Iod-Ethamine, in doses of 1 gm. per kgm. body weight, upon the concentration of cold-alcohol-insoluble, or "protein," blood iodine of rabbits.

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OBSERVATIONS ON VARIOUS TYPES OF MOTION CAUSING VOMITING IN ANIMALS¹

BY R. L. NOBLE²

Abstract

Extensive observations on the motion factors causing vomiting in animals are reported. Using a standard procedure of swinging, 81.5% of 70 dogs were found to be susceptible to motion. Cats were highly resistant to motion sickness and did not show an increased susceptibility after treatment with prostigmin.

Susceptible dogs were divided into Groups 1, 2, and 3, of increasing susceptibility, by means of determining their response to swinging through angles of 90°, 45°, and 22½°. The percentages of animals found to be susceptible in these groups were 24, 40, and 36, respectively. Swinging the animal in a sideways position caused a reduction in the number being sick but the relative susceptibility of the three groups was unchanged.

Exposure to the motion of a motor truck and a boat in rough weather caused 10 of 16 animals to be sick—seven being seasick. The dogs that vomited were not those most susceptible to swinging but were evenly distributed through the three groups.

Animals have been exposed to the separate component motions of the swing and the effectiveness of the different motions compared. Horizontal motion is the most effective per se. The vertical component has some effect and the angular, probably none. No one component motion was as effective as the composite motion. The rate of the horizontal motion was an important factor per se, an increased effect being caused by an increased frequency. The effectiveness of vertical movement could be increased by increasing the distance traversed. Increasing the rate was ineffective.

Swings with six different lengths of radii and swinging through three different angles are reported. The optimal length of radius appeared to be about 14 ft., longer or shorter radii causing a falling off in susceptibility. Reducing the angle of swinging caused a lower incidence of vomiting except where a very short radius was used. In this case an increased response occurred, probably related to the increase in rate of swinging.

Attempts to obtain a more effective type of motion by using special apparatus are described. There was no evidence that these efforts improved the results obtained on the swing.

Until the start of the war little use had been made of experimental animals in the study of motion sickness. Although many species of animals were known to be susceptible to motion Pozerski (2) in 1921 studied the effect on dogs. By using a simple apparatus that gave rise to a vertical and lateral motion he was able to cause dogs to vomit. He noted that if the animals had been fed recently the symptoms were increased. In 1931 Sjöberg (3) made a more detailed study of motion sickness. He was able to cause vomiting in dogs when they were subjected to the motion of an express elevator or when elevated in a crane. He believed the vertical movement was the most important factor in causing illness. In other experiments he noted that section of the eighth nerve or destruction of the labyrinth rendered the animals

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² Assistant Professor.

immune. The study of motion sickness in animals was commenced in this Institute in the summer of 1942 in order to test therapeutic agents and to investigate the causative mechanism.

This paper, the first of a series, will describe various experiments designed to study the response of cats and dogs to different forms of motion so that methods for assaying therapeutic measures could be formulated. Since the dogs used for these experiments have been from a colony of susceptible animals maintained for some years, it is necessary as an introduction to give a brief résumé of the conditions of the research over this period. When the work was begun in 1942 it was most convenient to use cats as experimental animals. The response of this species to the motion of a simple swing was found to be unsatisfactory because the incidence of sickness was low. Dogs were then introduced in the experiments and found highly satisfactory. As the pressure of this research increased an electrically driven swing, similar to those being used for testing humans, was erected. A colony of some 25 to 30 susceptible dogs was established and has been maintained, so that these animals have been tested at weekly intervals during the last three years. This colony has been used for testing various drugs, as will be described in a subsequent paper, or has been used in the experiments that will be given in detail in this paper. Certain factors that affect the dogs' response to motion have been established and will be reported in further papers, but in order to assist in evaluating the results to be presented here, they may be briefly summarized. Dogs have been found to vary greatly in their absolute susceptibility to motion. Certain highly susceptible animals give extremely consistent results and over a period of years do not become adapted to motion. Such animals may safely be used twice a week without altering their control response. Other less susceptible animals show a greater variation in response and gradually become adapted to motion. This adaptation may be very slight and gradual or be marked and come on after only a few exposures to motion. Reflex vomiting from conditioning has not been seen though animals have been swung regularly for three years. The dogs used in the experiments to be described have been selected as animals that would give a consistent response to swinging. Many of them had to be used continuously for over a year before the present results were obtained. Although there is a gradual tendency for any dog to become adapted to motion the changes expected in these animals would be slight and would not appreciably alter the results reported.

Methods

The animals used in this study consisted of mongrel dogs and cats of both sexes and all ages. Tests were performed after a fasting period of 18 hr. but the animals were fed immediately before being subjected to motion. The motion was continued for 45 min. except where indicated unless vomiting took place. Vomiting and not salivation was used as the criterion of susceptibility. The animals were placed in tight fitting boxes with an opening for the head so that they could not turn around. No attempt was made to

fix the head since this resulted in struggling. Various types of apparatus have been used in order to give rise to different forms of motion. In most cases a simple swing has been used and in initial experiments the radius of the swing was kept constant but the angle of the arc through which the swing passed was altered. A reduction in the angle reduced the effect of g but kept the rate of swinging constant. Other experiments are described in which the radius of the swing was increased or decreased, and the arc of swinging was also altered. The motion of an ordinary swing was broken down into its component parts of a vertical, horizontal, and angular motion. The effects of these were compared when tested separately. Other experiments were designed to give rise to more bizarre types of stimuli, and the apparatus will be described under the appropriate heading. Finally, in order to induce a completely different type of motion, 16 animals were taken by motor-truck to a lake where they experienced 45 min. in a small launch on rough water. The details of these various experiments will be described with the results. The first experiment on the effect of different types of motion was started in 1943, and a group of 16 susceptible dogs was used. These animals are those described in the initial part of the paper and the results on individual animals are shown in Table I. Some months later a more extended series of observations were made using a group of 19 dogs. Of these animals 13 had been used in the initial study. These results have been summarized in tables and the experiments have been reported in chronological order.

Results

Susceptibility of Animals

In order to select animals for these experiments a series of cats and dogs were tested for susceptibility to motion. The animals were obtained directly from the general stock colony. As far as possible, dogs of either sex but under 15 kgm. were selected. The total incidence of sickness may be calculated from experiments using two different swings. In early experiments the swing consisted simply of a box to hold the animal, supported from the ceiling by two ropes. The box was pushed by two assistants and kept so that the animal's long axis was in the same direction as the arc of the swing. The radius for this arc was 20 ft. and its distance approximately 26 ft. The animal completed 25 complete to-and-fro swings each minute. In some experiments the swinging was interrupted by five-minute periods of short, more rapid, jerky swinging, but this did not appreciably affect the susceptibility of the animals. These observations, therefore, are not recorded separately. Most of the animals tested were run on an improved type of electrically driven swing. This apparatus was modelled after that described by Major A. Cipriani (1). A platform was suspended from each corner by four supports running to a two point suspension from a common axle. Once the swinging motion had been started an electric motor, suitably geared, was able to maintain the swing at a constant arc. This could be read off on an indicator. The driving impulse of the motor came on intermittently with each

swing. Two boxes, placed horizontally side by side in the long axis of the swing were fixed to the platform so that two animals could be swung at the same time. The animals faced in opposite directions. The platform and animal boxes may be seen in the Figs. 1 and 2. The radius of this swing was $14\frac{1}{2}$ ft. and the rate of swinging was 15 complete swings a minute. For these tests the swinging was always through an angle of 90° . These conditions have been used for various tests and are referred to as the standard swing. Animals were swung either until they vomited or for a period of 45 min. Those going 45 min. were considered for the purposes of this experiment as being non-susceptible. In a few cases animals were stopped after 30 min. if they showed no salivation or untoward symptoms and were considered as non-susceptible.

Cats.—A total of 21 cats were tested. Of these only three vomited on their first exposure to motion but two of these failed to become sick on at least two subsequent tests. One cat only gave consistent results, vomiting in from 5 to 10 min. after the start of the swing. This animal was used satisfactorily for some 25 tests but after this time it developed the habit of lying on its back while being swung. This manoeuvre apparently rendered the stimulus ineffective since the animal did not become sick. In an attempt in early experiments to increase the susceptibility of cats, three animals were injected subcutaneously with 0.5 cc. of prostigmin (Roche), 1 in 4000, before swinging. This treatment did not cause them to become susceptible. The low incidence of motion sickness in cats made their use unsatisfactory for the purposes of these experiments so that subsequent experiments were performed with dogs.

Dogs.—A large series of dogs have been tested on the types of swings described. Of the first 70 dogs tested 57 vomited and only 13 were classed as not susceptible. This would indicate that under the standard conditions described 81.5% of dogs are susceptible to motion sickness. Most of the susceptible animals show increased salivation while being swung especially those that continue for the longest period of time. After being sick the dogs are quite normal and readily take food. Unless swinging is continued and the animals made to be sick repeatedly there is little symptomatic evidence that the dog finds the experience unpleasant. The time of vomiting varied from a few minutes to 45 min. after the start of the swinging. Some of those animals doubtlessly would not have given consistent results had they been tested repeatedly. In selecting dogs for repeated tests the more susceptible ones that vomited most rapidly were generally chosen. A colony of 25 to 30 susceptible dogs was selected and has been maintained for the various experiments to be described. During the extended studies three female animals have become pregnant and have been tested at weekly intervals up to one week before term. Pregnancy did not alter the susceptibility of these animals in any way. Another animal developed distemper with gastrointestinal symptoms and recovered after treatment. This dog appeared to have a lowered susceptibility to motion for some weeks after recovery.

Effect of Alteration of the Angle of Swinging

In experiments using the electrically driven swing it seemed of interest to attempt to measure the degree of susceptibility of the dogs and to divide them into groups according to their sensitiveness to motion. The simplest method of varying the amount of g and intensity of the swinging was to keep the radius of $14\frac{1}{2}$ ft. constant but to alter the angle and arc through which the swing passed. In the initial experiments the maximum angle of 90° was used as the standard swing. The effect of reducing this to 45° and $22\frac{1}{2}^\circ$ was therefore determined. Reducing the angle to $\frac{1}{2}$ and $\frac{1}{4}$ of the original 90° lowered the effect of g to $\frac{1}{2}$ and $\frac{1}{4}$, respectively, while the frequency of the swing remained constant. Reducing the arc of the swing would also cause a decrease in each of the component parts of the motion to be referred to later. Values for g with an arc of 90° were calculated as follows, from the report by Major Cipriani. Radial acceleration at the lowest point of the arc was $1.585g$; and at the end of the arc $0.707g$; the change in acceleration therefore was $0.878g$. In experiments using an angle of $22\frac{1}{2}^\circ$ it was necessary to aid in the control of the swing by pushing it by hand. In the first experiment started in 1943, 16 dogs were tested with the different degrees of swinging and are listed in Column 1 of Table I. The time at which vomiting occurred is noted and an N indicates the animal was not sick even though swinging continued for 45 min. The dogs were divided into Groups 1 to 3 of increasing susceptibility. These results show that dogs may conveniently be divided into groups of different susceptibility, depending on their response to reduced swinging. The method of doing this is indicated in the table and, for other types of experiments, it has been found of great value to classify all dogs in this manner. Of a total of 25 susceptible dogs tested in this way it has been found that 24% were of Group 1, 40% of Group 2, and 36% of Group 3. These figures, when considered in view of the preceding result that, of all dogs tested, 81.5% were susceptible, allow one to calculate, not only how many dogs may be susceptible, but also their degree of susceptibility. The times of vomiting, as seen in the first column of Table I, indicate that this is not a reliable indication of susceptibility per se. Dogs in Group 1 may vomit as rapidly as those of the other groups. When an individual animal is considered, however, it may be seen that in some cases there is a tendency for the time of vomiting to become increased as the intensity of the swinging is reduced. There is also a trend for the time taken to vomit to become increased when the values for reduced swinging are compared in Groups 2 and 3. It has been noted repeatedly that, if an animal takes 40 to 45 minutes to vomit, the stimulus is only just effective and vomiting will not occur if the degree of swinging is reduced. It is believed that although calculation of the average time of vomiting is of some value in certain experiments the best criterion for susceptibility is the percentage of animals that vomit from any given degree of stimulation. Data on the consistency of the results obtained on repeated

TABLE I

EFFECT OF DIFFERENT DEGREES AND TYPES OF MOTION AND CHANGE OF POSITION, IN INDIVIDUAL DOGS

Dog No.	Straight position			Sideways position			Vertical motion	
	90°	45°	22½°	90°	45°	22½°	4 ft. 2 in.	2 ft. 1 in.
<i>Group 1—slight susceptibility</i>								
25	12	N	—	N	—	—	N	—
42	25	N	—	N	—	—	N	—
48	22	N	—	N	—	—	N	—
59	20	N	—	N	—	—	N	—
27	10	N	—	16	16	N	N	—
<i>Group 2—moderate susceptibility</i>								
13	9	10	N	N	—	—	N	—
44	17	20	N	N	—	—	N	—
47	19	30	N	N	—	—	N	—
58	12	15	N	25	N	—	N	—
19	22	15	N	12	40	N	12	N
53	20	35	N	21	30	N	15	N
<i>Group 3—marked susceptibility</i>								
1	16	16	23	22	18	N	N	—
40	10	12	28	25	35	N	N	—
3	7	10	14	8	5	20	15	N
51	20	12	20	20	23	25	15	N
57	4	4	11	10	9	12	15	N

tests will not be given here, but it should be stressed that these animals had been used repeatedly and their usual response to the standard swing was well established.

Effect of Swinging the Dogs in the Sideways Position

Under the experimental conditions described it was possible for the dog to have some freedom of movement of its head. This would obviously alter the relative position of the semicircular canals to the direction of the swing. It was considered essential, therefore, to determine what the effect would be of placing the animal sideways across the long axis of the swing. The same dogs described in the preceding paragraph were therefore tested in this position and the results are listed in the second column of Table I. It may be seen that swinging the animals sideways definitely reduced the incidence of sickness. This was demonstrated for most of the dogs in Group 1 and for half of Group 2, even when the full swing through an arc of 90° was used. When the reduced swing of 45° and 22½° was used the same trend of results was obtained. It seems clear that the original grading of susceptibility could also be applied in a general way to the animals when they were swung in the sideways position, although the over-all susceptibility was appreciably less.

Effect of Separate Components of the Movement of the Swing

With the completion of the previous experiments it was decided to test the dogs to the separate types of movement that compose the complex movement of the swing. These may be briefly stated as being a vertical component, which by measurement in the swing through an arc of 90° was 4 ft. 2 in. Similarly for a 45° arc the drop was 2 ft. 1 in. The horizontal component for an arc of 45° was equivalent to 11.375 ft. The third movement was the turning around the central axle or the angular component. Apparatus was designed to test these three separate types of movement and may be described briefly. Vertical movement was obtained by fixing the box for the dog by two ropes that ran over two large pulleys and were attached to the two near uprights of the electric swing. By running the swing in its usual way the box was directly raised and lowered at the same rate as the swing and with the same driving control. Such an arrangement may be seen in Fig. 3. The swing was also used as the driving force for the horizontal movement. A two-wheel platform on which was fixed the animal box was run along the floor and fixed directly onto the base of the swing by connecting rods. In order to allow for the curve of the arc of the swing it was necessary to introduce a hinge at each end of the connecting rods, as may be seen from Fig. 4. With this arrangement it was only possible, because of lack of space, to run the swing at an arc of 45° . This gave rise to a back and forth horizontal excursion of 11.37 ft. running in time with the swing. An attempt was made to initiate the turning or angular component of the swing in the following manner. A metal axle was run through the top of the animal box so that the box could be rotated. The rotation through an angle of 45° was carried out by manually pushing on a handle in time with a metronome so that 15 complete back and forth movements took place every minute. This apparatus is shown in Fig. 5.

Vertical Movement.—These results have been included in the third column of Table I. It may be seen that with a vertical drop of 4 ft. 2 in., the same magnitude as that occurring with a swing through 90° , only five dogs vomited. With a reduction to half the vertical distance no dogs were sick. The susceptible animals were from those of greater susceptibility, Groups 2 and 3. The response to the stimulus from vertical movement is much less therefore than with the complete swing. The arrangement of the apparatus and method described would indicate that the results from the two motions should be directly comparable.

Horizontal Movement.—The effect of the horizontal component corresponding to swinging through an arc of 45° was determined on the dogs of Groups 2 and 3. Two dogs, Nos. 1 and 3, vomited after 40 and 42 min. respectively. This motion was also, therefore, less effective when compared with the complete swing. However, since the equivalent arc of the whole swing was only 45° , the horizontal movement was more effective than the vertical, as no dogs were sick with a vertical drop of 2 ft. 1 in. The relatively greater effectiveness of the horizontal component than the vertical will be confirmed in experiments found later in this paper.

PLATE I

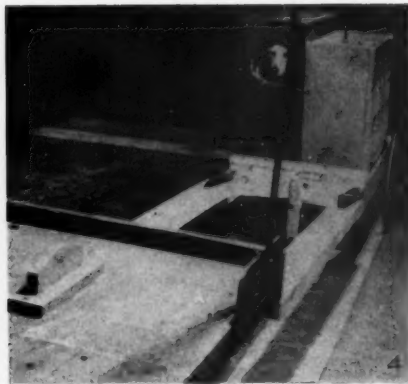
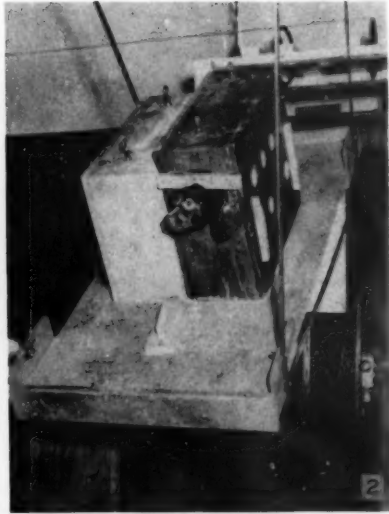


FIG. 1. *Standard electric swing.*
FIG. 2. *Standard swing at height of arc.*
FIG. 3. *Apparatus for vertical motion.*
FIG. 4. *Apparatus for horizontal motion.*

PLATE II

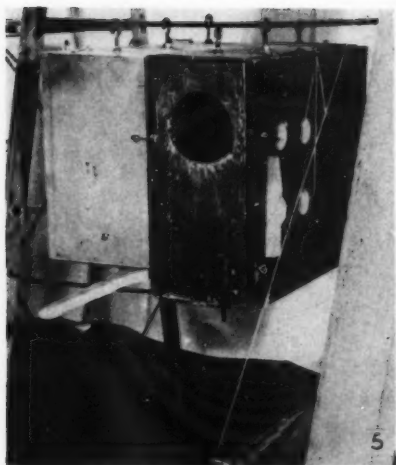


FIG. 5. *Apparatus for angular motion.*
FIG. 6. *Motor-boat experiment.*
FIG. 7. *Apparatus for 'washboard' motion.*
FIG. 8. *Apparatus for 'roller' motion.*

Angular Movement.—Only the dogs of Group 3 were tested using the angular component of the swing. No animals were sick after 45 min. of this type of stimulation. This factor would therefore appear to be the least effective of those described.

From a consideration of these results it is concluded that since no component motion of an ordinary swing is as effective by itself as the composite motion, the stimulation of the swing is probably a resultant of the three types of motion. Of the component motions the horizontal and vertical were effective but the latter was of less intensity; the angular was not effective per se.

Seasickness and Motor Sickness

Having determined the response to swinging and the different motion factors involved it was decided to expose the same group of dogs to other types of motion known to result in sickness in humans. Arrangements were made, therefore, to transport the 16 dogs in a Ford truck a distance of four miles to a lake and to take them in a motor-boat in rough weather. The dogs were tied in the truck and driven to the lake. The road to the lake was paved, mostly through the city, and the driver drove slowly there and back. The launch was an open one of approximately 30 ft. in length and 6 ft. wide. The dogs were tied around the side, some in the stern, and a few in a forward cockpit. They sat on the floor or on benches along the side, as may be seen in Fig. 6. Lake St. Louis, during the test, was moderately rough and choppy, with the direction of the wind and waves tending to shift. The launch was headed obliquely into the waves and run very slowly—this gave a predominantly rolling movement with some pitching. The maximum movement of the boat was probably of from two to three feet. The wind was strong and gusty and the temperature was 32° F.; most of the dogs became wet from spray. Because of the severe weather conditions the animals were only kept on the lake for 45 min. All the dogs had been fed before leaving the animal house.

Motor Truck.—Two dogs of Group 1, Nos. 59 and 27, vomited on the way to the lake from the motion of the truck. This occurred after 20 to 30 min. One of these dogs vomited again when in the boat. After being in the launch, and on the way home, two dogs from Group 2, Nos. 44 and 51, vomited in the truck. Neither of these dogs had been sick in the launch.

Boat.—During the 45 min. on the lake seven of the 16 dogs vomited. One dog, No. 19, vomited at least twice. The times of vomiting were 13, 14, 20, 23, 30, 35, and 44 min. after leaving the dock. Two dogs, Nos. 42 and 27, were from Group 1. Three, Nos. 13, 53, and 19 from Group 2, and two, Nos. 3 and 51, from Group 3. As mentioned above, dog No. 27 had previously vomited in the motor truck on the way to the lake. When the dogs were first in the boat they were lively and noisy, but after 10 min. they became quiet, and at the end of 45 min. they were subdued, dejected, and obviously miserable. One had the impression that they were definitely disturbed by the situation and the contrast to their condition after exposure to motion on the

swing was striking. It is believed that if the trip on the lake had been more prolonged all the dogs would have been sick. The total incidence of sickness in this experiment was four dogs in the motor truck, one of those and six others in the boat, giving a total of 10 out of 16 dogs being ill. Of these 10 dogs, three were from Group 1, five from Group 2, and two from Group 3. When these results are compared with those of the swing in Table I, it is apparent that there was no relationship in this single experiment between susceptibility to the motion of the swing and that of a motor truck and boat. One would have anticipated that the dogs of Group 3 would have been the most susceptible. The lack of correlation in susceptibility between seasickness and swing sickness has also been noted in human experiments and will be referred to in another paper.

Effect of Alterations in Length of the Radius of Swing

The experiments to be reported in the remainder of this paper were performed at a later date, from February to September of 1944, with an increased number of animals. Thirteen of the dogs used in the preceding experiments were again used but six new animals were also included. In all tests, therefore, 19 dogs were used except in special tests as indicated. The object of these experiments was to try to determine how small and what type of stimulus was most effective in provoking vomiting in the dog. From the preceding results it was obvious that the extent of the motion experienced by the animals in the truck or in the boat was of much less magnitude than that derived from the swing. It seemed reasonable, therefore, to anticipate that certain types of motion of small excursion might be found effective. In initial tests the effect of altering the radius of the swing was determined. Decreasing the radius of the swing resulted in an increase in the rate of swinging and a shortening of the distance of the arc through which the animal swung. The duration of the stimulus would also be decreased. For any fixed angle of swinging the force of g remains constant, since changing the radius does not alter the magnitude of the radial acceleration. In most cases different angles of swinging were tested with a fixed radius, as previously the angles chosen were 90° , 45° , and $22\frac{1}{2}^\circ$. To obtain radii other than $14\frac{1}{2}$ and $11\frac{1}{2}$ ft. the electric swing could not be used. Instead, the box was suspended by two ropes and the distance from the floor of the box to the fulcrum measured to obtain the radius. The box was pushed by hand and maintained at its natural rate of swinging—the angle for swinging was judged by tapes held at the required position on a screen beside the box. With the shortest radius of 1 ft. 10 in. the box was swung on an iron rod as described for angular motion in a preceding paragraph. In this case the box swinging freely maintained a rate of about 40 per minute. For this and the other rates of 15 and 30.5, as described, it was necessary to constantly push the box in time with a metronome. In all cases the position of the box and the dog were parallel to the axis of the arc of the swing. The results of this experiment are summarized in Table II. Since a tabulation of some 275 individual tests would be too cumbersome,

TABLE II
EFFECT OF DIFFERENT RADII ON SUSCEPTIBILITY

Radius of swing	Rate/min.	Distance of arc for 90°	Angle of swinging		
			90°	45°	22½°
			Sick and av. time of vomiting		
29 ft. 6 in.	13	38.4 ft.	—	69% (23)	16% (29)
14 ft. 6 in.	14.5	22.7 ft.	100% (16 min.)	90% (19)	37% (19)
11 ft. 6 in.	16	18.0 ft.	95% (18 min.)	—	—
7 ft. 3 in.	21	11.3 ft.	90% (21 min.)	79% (24)	37% (28)
3 ft. 1½ in.	30.5	5.2 ft.	85% (19 min.)	63% (23)	48% (28)
1 ft. 10 in.	15	3.4 ft.	0%		
	30.5		16% (19)		
	40		26% (20)		

only the average percentage of the 19 animals that vomited is listed. The average time of vomiting is also shown, enclosed in parentheses.

From the results noted for the standard swing with a radius of 14½ ft., it may be seen that 37% or 7 of 19 animals vomited when swung through an arc of 22½°. These would represent animals whose susceptibility would place them in Group 3 as previously described. With an angle of 45°, 90% or 17 animals were sick. By difference this would show that 10 or 53% were of Group 2, leaving 2 or 10% in Group 1. A number of conclusions may be drawn from a consideration of all the results tabulated. If the radius is shortened, with a consequent increase in rate, the susceptibility of dogs swung through 90° decreases. Eventually, with the shortest radius of 1 ft. 10 in. the stimulus is hardly effective. However, it is seen in this case that an increase in the rate from 15 to 40 swings per minute causes an increased incidence of sickness. The same effect of reducing the radius is seen when swinging is through an arc of 45°. Here, however, it appears that the maximum response is with a radius of 14½ ft. With a longer radius of 29½ ft. the incidence falls off. This relative ineffectiveness of a very long radius is again shown for an angle of 22½° where only 16% of the dogs were sick compared to 37% when the radius was shortened to 14½ ft.

These results would indicate that an optimum length for the radius exists and is of the order of 14½ ft. Longer or shorter radii cause a falling off in the incidence of sickness. The exception to this appears to be with a short radius of 3 ft. 1½ in. with swinging through 22½°. For some reason this gave rise to an increased response. It seems likely that with the smallest arc of 22½° the movement is chiefly a horizontal one, and in this case the frequency of stimuli seems to be the predominant factor. Another consideration is that at a rate of 30.5 the dog receives twice the number of stimuli per minute than at a rate of 15. If one considers the number of stimuli required to make the dog sick rather than the incidence for a fixed time of swinging, the results are somewhat altered. The smallest number of stimuli in any of the tests were

received by dogs swung at the lowest rate of 13 per minute. An animal not sick in 45 min. would therefore receive 585 swings whereas an animal swung at 30.5 per minute would receive this number of swings in 17 to 18 min. In the actual experiments at a rate of 30.5 only 10% of the dogs were ill after a comparable number of swings. If one considers these findings in view of the preceding ones it seems that with frequencies between 15 and 30 the greatest effect is found with the greatest arc of swinging. For any given angle where g remains constant despite alterations in radii, the differences in effect are probably directly related to alteration of the vertical and horizontal components of the motion. With the smallest arc of swinging the vertical component is very small and the horizontal motion very short, but in this case the increased frequency gives rise to an increased number of briefly acting stimuli. The importance of the rate of swinging may be seen in the following section of the paper.

The Effect of Horizontal, Vertical, and Angular Movements

As in the first group of dogs tested the component parts of the motion were tested on the dogs separately. The 19 animals used, therefore, were the same as those used for the data listed in Table II. The vertical motion for frequencies of 15 per minute was produced, as previously described, with the swing as the driving force. A vertical drop of 4 ft. 2 in. was used as previously, and also one of 8 ft. 4 in. Where a frequency of 30 per minute was used the box was moved through a vertical distance of 2 ft. 1 in. by manual force. To obtain the horizontal movement at higher frequency it was not possible to use the swing as a driving force. The box was suspended, therefore, from ropes 29½ ft. long and pushed manually through a distance of 2 ft. 10 in. at a frequency of 15 or 30 per minute. The vertical component in this motion was negligible and the motion was therefore considered as purely a horizontal one. One new type of apparatus was constructed. This consisted of a four pole swing in order to compare the results with those obtained with horizontal motion. The length of the four ropes was 5 ft. and the box was pushed through a distance of 2 ft. 10 in. by hand at rates of 15 or 24 per minute. The latter was the natural rate of swinging. A guiding rod was placed along the side of the boxes to prevent their turning sideways. The motion resulting with this type of suspension is essentially a horizontal one with only a slight vertical component of a few inches. The results of these experiments, and those for angular motion from Table II, are listed in Table III.

These results confirm those of the previous experiment listed in Table I but also show the importance of the rate of stimulation. The vertical motion may be seen to be only slightly effective even when the rate is doubled to 30 per minute. In each case the same dog was sick. Increasing the vertical drop to 8 ft. 4 in. increased the incidence so that three animals were sick. The two types of horizontal motion gave essentially similar results. Such a form of motion is relatively effective especially when it is noted that the distance of the motion is only 2 ft. 10 in. The enhanced effect of increasing

TABLE III
EFFECTS OF VERTICAL, HORIZONTAL, AND ANGULAR MOTION

Type of motion	Excursion of box	Rate per minute	Percentage sick in 45 min.
Vertical	2 ft. 1 in.	30	5
Vertical	4 ft. 2 in.	15	5
Vertical	8 ft. 4 in.	15	16
Horizontal	2 ft. 10 in.	15	0 (5% in twice control time)
Horizontal	2 ft. 10 in.	30	37
Horizontal-4 pole	2 ft. 10 in.	15	5 (5% in twice control time)
Horizontal-4 pole	2 ft. 10 in.	24	37
Angular	3 ft. 3 in.	15	0
Angular	3 ft. 3 in.	30.5	16
Angular	3 ft. 3 in.	40	26

the rate is definite in each case. To determine whether the total number of stimuli received was the factor causing sickness, each animal was swung at the lower rate for at least twice the time it took to vomit at the higher rate. This did not give rise to an appreciably different incidence. In these cases, therefore, the increased frequency is the factor causing the increased number of animals to vomit. The effects of angular motion have been taken from Table I and again show that a more rapid rate of swinging causes increased susceptibility.

Other Types of Motion

A number of experiments were conducted by swinging the animals under different kinds of conditions, and with unusual types of motion. These were designed to attempt to obtain the maximum response with the smallest degree of motion. Two experiments were designed to introduce a rapid, small vertical component into a horizontal motion. In the first of these a cement base with a corrugated surface was constructed and the two wheel carriage with the box was pushed back and forth over it. This apparatus is shown in Fig. 7. This set-up caused a very bumpy horizontal motion although the vertical component was only approximately two inches. Since no springs were employed the dog received considerable jarring and may have been disturbed by the noise. The carriage was pushed through a distance of five feet over this washboard type of surface at a rate of 15 per minute. The eight most susceptible dogs were tested by this method. The same type of motion was obtained by suspending the box by two soft springs of approximately 2 ft. in length. The radius for the experiment was $7\frac{1}{4}$ ft. and the box was pushed through an angle of 45° , at a rate of 21 per minute. At each excursion the operator pushed down on the box to give it a combined horizontal and springy vertical motion. All the dogs were tested by this means and those that became ill were tested the following week using the same degree of horizontal motion without any springing. The times of vomiting were then compared.

To determine whether upsetting the balance of an animal would affect his susceptibility it was arranged to make the box as unsteady as possible. To do this it was suspended by a single centrally attached rope, 3 ft. 1½ in. in length. As a result of the unequal weight distribution caused by the dog changing position the box tilted at various angles. The operator also tilted the box while pushing it through an angle of 90° at a rate of 15 per minute. Six of the least susceptible animals were tested in this fashion. In a further experiment it was decided to suspend the animal in slings so that its feet did not touch the ground. Three animals were tested in this way using the full electric swing through an angle of 90°.

An apparatus was constructed to alter the downward pull of the ordinary swing but to keep a similar kind of motion. To do this the box was suspended from an axle at the top end of two upright poles. An axle was run through the centre of these poles so that they would describe a complete circle on it. The bottom end of the poles was fixed with a suitable counterweight for the dog and box, as may be seen in Fig. 8. By pushing the box back and forth the animal was made to move through an arc of 90° but in this case the motion was through the top part of the circle. The radius used was 3 ft. 1½ in. and a rate of 18 per minute was maintained. Ten of the most susceptible animals were subjected to this motion, referred to in the table as 'roller.'

The experiments described were run in the order tabulated. At the end the 19 animals were run in the electric swing through 90° to be certain that none became adapted. This control run is therefore included in Table IV with the results of the above varieties of motion.

TABLE IV
EFFECT OF DIFFERENT TYPES OF MOTION ON SUSCEPTIBILITY

Motion	Angle of swinging,°	Radius, ft.	Excursion of box	Rate/min.	Type and no. of animals	Percentage sick
'Washboard'			5 ft.	15	High susceptibility—8	13
'Springs'	45	7 ft. 3 in.	5.6 ft.	21	All	42 (18 min.)
Control—no springs	45		5.6 ft.	21	All	42 (13 min.)
One rope and tilting	90	3½ ft. 1½ in.	2.6 ft.	15	Low susceptibility—3	0
'Slings'	90	14½ ft. 6 in.	22.7 ft.	14½	High susceptibility—3	100
'Roller'	90	3 ft. 1½ in.	2.6 ft.	18	High susceptibility—10	40
Final control, electric swing	90	14 ft. 6 in.	22.7 ft.	14½	All	100 (15 min.)

The results of the modified types of motion may be seen in Table IV and can be compared with the corresponding degree of ordinary swinging listed in Table II. In general the attempts to increase the effectiveness of stimu-

lation were not successful. The introduction of an uneven or bumpy type of horizontal movement was ineffective, as was the attempt to upset the balance of the dogs in the single rope experiments. Even the average time of vomiting was not reduced in the 'spring' experiment. That stimulation arising from the feet is of no importance is substantiated by the dogs that vomited even though held off the floor by slings. The roller causing the dog to swing through the inverted type of arc was quite effective. This may suggest that the otolith organs are of little importance as the originator of stimuli that come from the labyrinth. The final control test showed that the animals had all remained susceptible throughout the experiments. The average time of vomiting of 15 min. checked closely with the figure of 16 min. seen in Table II and obtained at the start of the observations.

Acknowledgments

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METHODS OF ASSAYING MOTION SICKNESS PREVENTIVES ON DOGS¹

By R. L. NOBLE²

Abstract

The details of assay methods used since 1942 to test the effectiveness of motion sickness preventives in dogs are described.

Animals first were classified according to their susceptibility to motion. Over a three year period dogs of moderate or marked susceptibility show little variation in the response to swinging or to treatment. Dogs of low susceptibility may become adapted to motion and be effectively treated by reduced dosage when used repeatedly.

All dogs were treated using V-12 ethyl- β -methylallylthiobarbituric acid as a standard. Complete protection followed oral doses ranging from 1.25 to 30 mgm./kgm. and no untoward side effects were produced. Dogs of lowest susceptibility were most readily protected—by doses up to 5 mgm./kgm. Moderately susceptible animals required up to 15 mgm./kgm. and markedly susceptible ones more than this dose.

Comparative assays with two other barbiturates showed that the results were not consistent when tests were made on groups of dogs of different susceptibility. V-12 was relatively more effective when tested on dogs of greatest susceptibility. Assays on dogs of low susceptibility that are readily treated are probably of questionable accuracy.

Reducing the degree of swinging by one-half allowed the dogs to be effectively treated by one-fourth of the former dosage. The relative difference in potency between V-12 and another barbiturate was reduced by the change in magnitude of the swinging.

The size and amplitude of the swing has a marked influence on quantitative assays. The smaller the degree of motion, the greater the apparent potency of a drug. Individual susceptibility to motion of the dogs is also a factor in obtaining comparable results. The greater the susceptibility, the more accurate the determination and comparison. Such factors will markedly limit comparisons of results obtained by different workers.

In a previous paper some of the factors that affect the production of motion sickness in experimental animals were described (1). As a continuation of this work methods for assaying drugs to be used as motion sickness preventives have been developed. The necessity of such methods was apparent in 1942 when it was found that certain compounds possessed the property of protecting dogs against sickness. In efforts to find the most effective drugs some comparative form of testing was necessary. The methods that were developed were influenced by certain conditions dependent on the war. In the first place it was of primary importance to find the most effective drug as quickly as possible rather than to compare accurately the potency of different compounds. As a result, new substances were compared with the most active one then available. If they were less active no attempt was made to determine their degree of activity. A large number of compounds were available for testing and it was impossible to obtain highly accurate results for all of

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² Assistant Professor.

them. The largest number of dogs that could be maintained for testing was between 20 and 30 and it was possible to use many of these animals only once a week because of the development of adaptation to motion. It was not practical, therefore, to use large numbers of animals for each drug. For practical reasons only the oral activity of the various drugs has been tested.

This paper refers only to the different methods that have been used for comparing various substances. In a subsequent paper the effects of some 150 compounds will be compared. As this work continued it became advisable to adopt one substance as a standard and compare others in terms of it. Experiments of this type will be described using the barbiturate, ethyl- β -methylallylthiobarbituric acid (V-12), as a standard. This compound was selected since it has been used for extensive animal tests and human experiments.

The study of the effects of drugs on motion sickness was commenced in this Institute in 1942. It was believed that it might be possible to find a barbiturate or related compound that, through a central action, might inhibit or depress the brain centres involved in the nervous reflex set up by motion. The general central depressant action of such compounds and the more specific action of certain hydantoins suggested that some substance might possibly exert a specific effect in motion sickness. Initially, therefore, it was decided to test barbiturates that had low hypnotic potency, since sedation would be an undesirable feature in treating military personnel. Through the early co-operation of Dr. Volwiler and Dr. Tabern of the Abbott Laboratories, and more recently through Dr. Chen of Eli Lilly and Company, a large number of barbiturates have been synthesized and donated for research purposes. Tests on these compounds will be published in due course. In early experiments it became apparent that a great number of barbiturates possessed the property of preventing motion sickness in susceptible dogs, without any undesirable side effect. Furthermore, this effect was not related to the hypnotic or depressant potency of the compound and appeared to be a specific type of action. Tests with a number of these compounds have been conducted on humans by various workers. In order to maintain secrecy and designate the type of experiment, any barbiturate tested on humans was given the initial V and a number. (It may be pointed out that the popularity of this initial was not anticipated when first used in 1942, and the designation V was selected to stand for vomit). Three barbiturates have been used in comparative studies for the development of an assay method and will be referred to in this paper.

Methods

Susceptible dogs were subjected to the standard swinging as described in a previous paper (1). The swing was electrically driven and had a radius of $14\frac{1}{2}$ ft. The frequency was 15 complete swings per minute and the angle through which the swing passed was 90° (except in one experiment, as indicated, where the one-half swing of 45° was used). Animals were used at four- or seven-day intervals so that the adaptation to motion that occurs

would be minimal. The susceptible dogs were subdivided into groups according to their susceptibility to motion, as previously published. Group 1 was the least susceptible, and Group 3, the most susceptible.

All animals were fasted for the preceding 18 hr. but were fed immediately before being swung. Most compounds were given in a small piece of meat or in capsules, from two to four hours before the test. Vomiting was used as the only criterion of susceptibility and those animals going 45 min. without being sick were considered as protected. In certain experiments animals that vomited after 40 to 45 min. were considered as benefited or improved.

Results

Consistency of Response to Swinging

In order to use the same dogs for testing over prolonged periods it was necessary to be certain that their response to swinging did not change. In early experiments, therefore, the dogs were tested from time to time without treatment. In most cases there was little evidence of any change in susceptibility after the first few swings. A few dogs, however, did develop a resistance after repeated tests at weekly intervals. It was noted that these animals were ones that had required 35 to 45 min. of swinging before being sick and were therefore only just susceptible at the start. From observations over the past three years it appears that all animals show a gradual reduction of the response to swinging. This is usually slight and may be shown only by the longer time required to make the animal sick. In most cases it is not of great enough magnitude to cause an animal to become non-susceptible. To avoid these changes only dogs exhibiting a rapid and marked response to motion have been selected for prolonged experiments. Recently, observations have been made on the change in response to treatment that occurs in dogs of Groups 1, 2, and 3, of different susceptibility. A group of six dogs of Group 1—indicating slight susceptibility—were tested with a dose of 1.25 mgm./kgm. of V-12 and one animal only was protected. After 24 tests at weekly intervals it was found on repeating this dose that five animals were protected. Furthermore, two dogs did not become ill on repeating the control test. This change was due to adaptation to the repeated swinging over this period. Of six dogs in Group 2, of moderate susceptibility, only one animal showed an altered response to a fixed dose of V-12 even though 37 tests at weekly intervals had been performed in the interval. Dogs of Group 3, of marked susceptibility, have been used twice weekly and after 35 tests did not show any variation in response to treatment. Some of the earliest dogs used in these experiments have been tested on a great many occasions, in most cases after treatment by drugs. Two examples may be mentioned. Dog No. 3 has been swung on 148 occasions since August, 1942, and has always remained in Group 3. Dog No. 1 has been swung 141 times. Originally of Group 3, it has become somewhat more resistant and is now of Group 2. These two dogs have vomited on 117 and 92 occasions respectively without showing any signs of conditioning. In 10 control tests over the three year

period the average times of vomiting were 12 ± 3.7 and 13 ± 6.1 min. In tests with compounds that were non-effective and that may be considered as controls Dog. No. 3 vomited 55 times at an average time of 13 ± 4.5 min. and Dog No. 1 was ill 57 times at an average time of 17 ± 5.2 min.

It seems obvious, therefore, that with proper precautions dogs may be selected so that changes due to adaptation to motion or other factors will not affect their use for assay purposes. Animals of low initial susceptibility may be expected over a period to become adapted to motion. If they are used for comparing the effects of treatment, frequent control tests should be performed.

Response of Dogs of Different Susceptibility to Treatment

After the first few compounds had been tested and compared it was noted that certain dogs were consistently protected, whereas others were little affected. It was obvious, therefore, that a difference in the response to treatment existed and was quite pronounced. It seemed likely that the least susceptible animals were responding best to treatment and vice versa. By dividing the dogs into groups of different susceptibility it was possible to test this hypothesis. A number of compounds of approximately the same potency had been tested on 16 dogs. These animals were those listed in Table I of the previous paper (1) and their response to treatment is given in Table I.

TABLE I
EFFECT OF BARBITURATE TREATMENT ON DOGS OF DIFFERENT SUSCEPTIBILITY

No. of dogs	Susceptibility	Group	No. of tests	Percentage of tests		
				Protected	Benefited	Negative
5	Low	1	38	82	13	5
6	Moderate	2	40	48	32	20
5	High	3	30	20	50	30

These results clearly show that the susceptibility of an animal to swinging has a direct relationship to the response to treatment. As would be expected the Group 1 dogs are readily protected by therapy whereas Group 3 are more resistant. The animals classed as benefited were those in which the time of vomiting was prolonged. It may be noted that treatment tends to raise the number of animals in this class, especially in Group 3 dogs. Apparently the dosage used had some effect but not enough to prevent vomiting. It has been found in these cases that only a small increase in the dose is necessary to obtain protection. In such experiments the time of vomiting is a useful guide for the conduct of further tests, but as previously pointed out, it is not entirely reliable if used as an index of susceptibility per se.

With such differences in the effects of treatment in the different groups of dogs it is readily appreciated that in comparative tests it would be necessary to use a large series of animals. Using only five animals it would be possible

to obtain values one to six times too high for the same compound, depending on the selection of the dogs. Whereas this danger was recognized in early assay tests, it was difficult to overcome, since a large number of drugs had to be tested and large numbers of dogs could not be used. In order to try to neutralize the effect of the wide difference in response it was decided to consider dogs of Group 2 to represent the average. The desired dose for treatment was therefore given to this group. If a dog of Group 1 was included in the test it was given only one-half the dose for dogs of Group 2. Similarly, dogs of Group 3 were given double the dose. This procedure, although not satisfactory, tended to give more consistent results, and assays by this method will be included in another paper. It appeared that the best method of assay would be one where the response of the dogs to some standard could be determined and other compounds then compared in potency. After the use of V-12 in human tests it was decided to adopt this compound as a standard for experimental work and the subsequent part of this paper will report these results.

Response to V-12 as a Standard

The effect of treatment with various doses of V-12 (ethyl- β -methylallylthiobarbituric acid) was determined on 16 dogs. The complete results are listed in Table II.

TABLE II
EFFECT OF TREATMENT WITH V-12 ON INDIVIDUAL DOGS

V-12 dose, mgm./ kgm.	Dog No.						% Pr.	Dog No.						% Pr.	Dog No.				% Pr.	Pr. total, %
	25	58	67	27	51	42		19	40	53	68	1	37		69	3	70	57		
	Time of vomiting, min.*							Time of vomiting, min.*							Time of vomiting, min.*					
0	15	30	40	40	10	15	0	9	8	8	18	12	12	0	23	10	10	5	0	0
1.25	NS	37	30	45	40	17	16													6
2.50	NS	NS	NS	30	40	38	50													19
5.0	NS	NS	NS	NS	NS	45	83	15	13	30	12	26	13	0	15	5	12	6	0	31
10.0	NS	NS	NS	NS	NS	NS	100	NS	NS	NS	NS	45	36	66	26	12	15	8	0	62
15.0												NS	NS	100	17	10	9	10	0	75
20.0															NS	NS	30	7	50	87
25.0																	NS	15	75	94
30.0																	NS	100	100	100

* N.S. indicates that sickness did not occur in 45 minutes.

The body of the table contains the time of vomiting in minutes for each test on the individual animals, NS indicates that sickness did not occur in 45 min. The dogs are listed so that the ones on the left-hand side of the table are the most readily treated and vice versa. It may be seen that the effective dose range for V-12 was from 1.25 to 30 mgm./kgm. The over-all percentage of dogs that did not vomit was listed on the right-hand column. The response of these animals to V-12 enabled one to divide them into three

groups, as indicated in the table. The first group were those protected by doses up to 5 mgm./kgm. The second group were those unaffected by 5 mgm. but protected by 15 mgm./kgm., and the third group not affected by 15 but protected by doses above this up to 30 mgm./kgm. The percentage of animals protected in these individual groups is listed in separate columns in the table. The animals in the groups classified in this way were tested and divided into Groups 1, 2, and 3 according to their sensitivity to reduced swinging, as previously described. It was found that in all cases except Dog 42 the groups corresponded exactly. This one animal is included in the first group in Table II as, although it was not protected by 5 mgm./kgm., it vomited at 45 min. or just at the end of swinging. It may be stated, therefore, that dogs may be classified according either to their susceptibility to graded amounts of swinging or to their response to treatment with V-12 after a constant degree of swinging. Animals of Group 1, indicating slight susceptibility to motion, are successfully treated by 5 mgm./kgm. of V-12 or less. Group 2 dogs, of moderate susceptibility, require 15 mgm./kgm. for successful treatment, whereas the most susceptible dogs—Group 3—require doses above 15 mgm./kgm. to prevent vomiting. It may be stressed that the doses of V-12 used in these tests did not cause any demonstrable side effects. A dose of 30 mgm./kgm. is the upper limit that can be used without consistently inducing undesired reactions. After such a dose sensitive animals may show a slight transitory unsteadiness on walking.

Comparison of V-1 and V-16 with V-12

Having determined the individual response of all the dogs to V-12 it was then necessary to see how they would respond in comparative tests with other barbiturates. For these tests two compounds were selected, V-1 (ethylallylthiobarbituric acid) and V-16 (dicrotylbarbituric acid). The response to treatment of the three groups of dogs was therefore determined and the average results are summarized and compared with V-12 in Table III.

If one considers the results obtained with Group 1 dogs it is seen that V-1 and V-12 were equally effective, whereas V-16 was approximately twice as active as V-12. With the Group 2 dogs, however, V-1 was definitely less effective than V-12. V-16, while still more active than V-12, was less so than in the preceding test. With the Group 3 dogs, V-1 was definitely less effective than V-12, and V-16 was not effective in a dose of 15 mgm./kgm. With 20 mgm./kgm. all the dogs were protected—this result has been placed in parentheses, since all the animals showed mild side effects. In other experiments it has been found that practically any barbiturate, if administered in sufficient dosage to give rise to side effects such as hypnosis, incoordination, and ataxia, will completely protect an animal against motion sickness.

The results in Table III are of interest since they indicate that in comparative tests divergent results may be expected depending on the susceptibility of the dogs tested. Apparently the curves for protection show different slopes, especially in the higher dose region. Because the variation in response

TABLE III
COMPARISON OF TREATMENT WITH V-12, V-1, AND V-16

Dose, mgm./kgm.	Percentage protected		
	V-12	V-1	V-16
<i>Group 1—six dogs—low susceptibility</i>			
1.25	16	—	50
2.5	50	50	—
5.0	83	—	100
10.0	100	100	—
<i>Group 2—six dogs—moderate susceptibility</i>			
5.0	0	0	16
10.0	66	0	82
15.0	100	82	100
<i>Group 3—four dogs—high susceptibility</i>			
15.0	0	0	0
20.0	50	0	(100)
25.0	75	—	—
<i>All dogs</i>			
1.25	6	—	19
2.5	19	19	—
5.0	31	—	44
10.0	62	37	69
15.0	75	69	75
20.0	87	75	(100)

seemed greatest when using Group 1 dogs and since these animals are the most likely to show changes due to adaptation, it is the custom at present not to use such dogs for assay purposes. It is believed that for practical purposes more accurate results can be obtained by using only dogs of Groups 2 and 3. Any susceptible animal, therefore, that is protected by a dose of 5 mgm./kgm. of V-12, is not used for assay tests such as will be described in a subsequent paper. The usual procedure at present is, therefore, to select dogs of Groups 2 and 3, having determined their response to V-12. Unknown compounds are compared with V-12 directly, using either group of dogs.

Effect of Reduced Swinging in Comparative Tests

Since the relative potencies of V-12 and V-1 were found to be different in dogs of Groups 1, 2, and 3, it was believed of interest to see what the relative values would be if the dogs were swung through an angle reduced to 45°. As previously shown, this reduced the incidence of sickness so that only dogs of

Groups 2 and 3 were affected; the change due to g being only one-fourth of that when the full swing was used. Since the stimulus due to the motion was obviously reduced it was anticipated that successful treatment of the dogs would be accomplished with a relatively low dosage. The dose of *V-12* for comparison was therefore one-fourth of the minimum effective dose determined for the full swing. The same dose of *V-1* was then compared in dogs of Group 2. These results are shown in Table IV.

TABLE IV
COMPARISON OF TREATMENT WITH FULL AND HALF SWING

	Dose, mgm./kgm.	Percentage protected	
		<i>V-12</i>	<i>V-1</i>
Full swing	10	66	0
	15	100	82
Half swing	$\frac{1}{4}$ M.E.D. for <i>V-12</i>	66	50

The values for *V-12* and *V-1* obtained on the six dogs of Group 2 may be compared with the full and half swing. Control tests with the half swing showed that all the dogs vomited in an average time of 25 min. It may be seen that the relative difference shown by the testing on the full swing between *V-12* and *V-1* is very definitely reduced when the half swing is used. In this case the two compounds were of approximately equal effect. This result, indicating equal potency, is the same as when the comparative test was made with dogs of Group 1, but with the full swing. When the half swing is used, however, dogs classified as Group 2 on the full swing would become of Group 1, were they to be re-classified in the same way but using the half swing. Reducing the effectiveness of the stimulus from swinging therefore causes an alteration in the comparative response of *V-12* and *V-1*. It is of interest to note that a reduction in g by one-fourth allowed the dogs to be treated almost as effectively as previously by a dose of *V-12* also reduced to one-fourth. The importance of using dogs of known susceptibility for assay purposes and the necessity of using a stimulus of fixed magnitude is quite obvious. Results obtained by different workers, therefore, are unlikely to be comparative quantitatively since the conditions, as described, that may affect the test will undoubtedly be different in separate laboratories. In general, anyone using a swing with dimensions smaller than those that have been described or using dogs selected at random would undoubtedly obtain different results. In such a case the dogs would be protected more easily and all drugs tested would appear more active. Differences in activity between compounds would be less when compared with the results reported in this paper.

Acknowledgments

This research was supported by grants from the Associate Committee on Army Medical Research of the National Research Council, Ottawa. Mr. E. Pedersen rendered valuable technical assistance. The author would like to thank Dr. J. B. Collip for his continued interest. The co-operation of the Abbott Laboratories in supplying the barbiturates described in this paper is greatly appreciated.

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A HUMAN EMBRYO OF TWO TO THREE PAIRS OF SOMITES¹

BY RALPH F. SHANER²

Abstract

A description is given of a human embryo with two to three pairs of somites and about 25 days old. In general the embryo tallies with the few other human embryos of the same stage hitherto described. It has a well developed head fold, but no tail fold. Its neural groove is everywhere open. There is a minute neurenteric canal, a long primitive streak, and long cloacal membrane. The last is degenerate in its caudal part. The optic primordium is faint, but a clear otic placode is present. Between the two extends a neural crest, the earliest on record. A slight fore-gut is present, ending in an oral membrane. There is a long tubular allantois. The notochord is strap-like, issuing from a Henson's node and ending in a prechordal plate. Two well developed pairs of somites appear with the beginning of a third. The mesoderm is split to contain two body cavities, which join beneath the fore-gut. The yolk sac is partly covered with blood islands. There are two definite umbilical arteries and traces of umbilical veins. Solid strands of angioblastic tissue beneath the fore-gut are the only signs of a heart. Scattered indications of body vessels also occur.

Introduction

The recent discovery of several human embryos less than two weeks old has attracted so much interest as to make one forget that embryos from the rest of the first month are still few in number. A short description of one embryo with two to three pairs of somites and a comparison of it with others of the same age may still be of value.

The human embryo, the subject of this paper, was found clinging to the external uterine orifice at a routine professional examination. All relevant clinical data are given in the following note from the physician, Dr. J. Ross Vant:

"This young woman is in her twenties, pregnant for the first time, and her last period began March 6. Her cycle is approximately 31 days. She aborted April 16. I suppose she should have ovulated on or about the seventeenth day of her cycle, which would make the possible date of conception March 22. The age of the ovum would therefore be 25 ± 2 days."

To estimate the age of the embryo, Dr. Vant has followed Rock and Hertig (8), who put the ovulation date on the 14th day preceding the onset of the next period. In this instance ovulation would have taken place on the 17th day of a 31-day cycle.

The specimen as removed from the uterine orifice consisted of an intact chorionic vesicle loosely attached to a fragment of decidua. The vesicle was

¹ Manuscript received July 28, 1945.

Contribution from the Department of Anatomy, University of Alberta, Edmonton, Alta.

² Professor of Anatomy.

covered with villi except for one small area. After fixation in 10% formalin the vesicle was opened and an embryo found within, anchored by a small amount of magma. The embryo was taken out of the vesicle, several camera lucida sketches made of it, and it was then cut into 10μ transverse sections, which were stained with haematoxylin and eosin. The chorion was trimmed rather too closely to the umbilical stalk. This and a slight gash in the yolk sac are the only defects in an otherwise excellent series.

General Description

The general layout of the Vant embryo can be seen in Figs. 1 and 2. It has a well developed head fold, but no tail fold. The neural groove is everywhere open, and terminates at a distinct neurenteric canal. Behind the canal there is a long primitive streak and still longer cloacal membrane. The only specialized parts of the yolk sac are a fore-gut and an allantois. A strap-like notochord is embedded in the roof of the yolk sac from Henson's node to the tip of the fore-gut.

The maximum length of the Vant embryo, measured along the curves of the sagittal reconstruction (Fig. 2) from the brain tip to the end of the primitive streak, is 1.5 mm. Of this the streak is 0.41 mm. or 27%. The straight line length is 1.2 mm. of which the primitive streak is 34%. These measurements are offered with the customary apologies for technical distortion but are nevertheless in accord with those of comparable embryos. The two to three somite embryo of Ingalls (3) has a straight line length of 1.38 mm., of which Bartelmez and Evans (1) allocate 26% to the primitive streak. The two to three somite embryo of Wilson (11) has a straight line length of 1.64 mm. of which the primitive streak is 29%. The two somite embryo of Ludwig (4) has a maximum length of 2.4 mm. of which one-third is assigned to the primitive streak.

The Vant embryo has a deep concavity instead of the tail fold found in other embryos of the same age. I suspect the concavity is more than an artifact or simple variation. Nearly every section from the brain tip to the neurenteric canal shows at least one mitotic figure in the ectoderm; numerous sections just anterior to the first somite show as many as six figures. Behind the neurenteric canal, only eight sections show mitotic figures, and none more than two. The preservation and staining of the primitive streak and cloacal membrane suggest dying tissue. It is at least plausible that the embryo had ceased to grow in this critical region and had been aborted in consequence.

EXPLANATION OF FIGURE

FIG. 1. Drawing, by Professor William Rowan of a wax model of the Vant embryo. Model $\times 180$, drawing $\times 80$. The dotted lines indicate the positions of the somites, otic placodes, and the neural crests. For other details compare with Fig. 2.

PLATE I



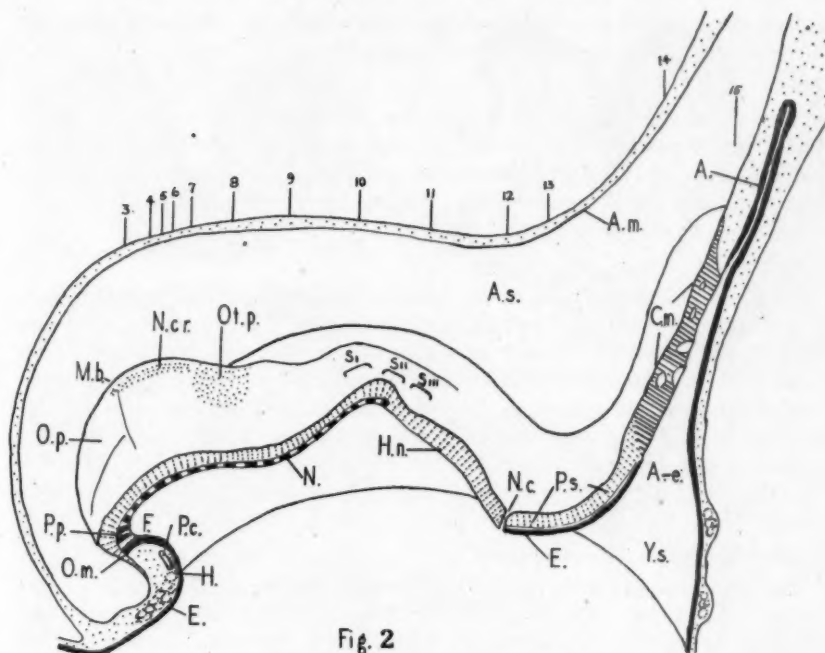


Fig. 2

FIG. 2. Sagittal graphic reconstruction. $\times 60$.

Key to abbreviations used in Figs. 2 to 20. Nos. 3 to 15, position of sections shown in Figs. 3 to 15; A., allantois; A.e., allantoenteric diverticulum; A.m., amnion; A.s., amniotic sac; C.m., cloacal membrane; E., entoderm; F., fore-gut; H., heart; H.n., Henson's node; M.b., midbrain bend; N., notochord; N.c., neurenteric canal; N.cr., neural crest; O.m., oral membrane; O.p., optic primordium; Ot. p., otic placode; P.c., pericardial cavity; P.p., prechordal plate; P.s., primitive streak; Y.s., yolk sac; S.I., II., III., somites; T.s., terminal sinus.

Special Structures

Neural Groove

The neural groove is everywhere wide open, as in other embryos of the same age. A casual inspection of the model (Fig. 1) might suggest that closure is imminent at the second somite. Mitotic figures are most numerous just before the first somite, and there is considerable cell division in the ectoderm throughout the somite area. But the lips of the neural folds are still far apart throughout this area (Figs. 10 and 11), and there is really nothing to contradict the general belief that closure of the neural groove begins between the third and sixth somites in slightly older embryos.

Eye

The anterior tips of the neural folds are slightly indented (Fig. 1), and are much thicker in section (Fig. 3) even when the effect of the plane of section is allowed for. These blunted tips are the optic primordia, according to Bartelmez and Evans (1). The left primordium bears a doubtful optic

sulcus, about as well developed as in the Ludwig embryo. The optic primordia lie definitely within the limits of the neural tube.

Ear

The otic placode appears as a lenticular thickening just outside the neural folds (Figs. 1 and 8). The placode is about as definite as that of the Ingalls embryo as shown in Fig. 30 of Bartelmez and Evans (1). There is a suggestion of a placode in the Wilson embryo. No otic placode is found in the comparable embryos of Ludwig (4) and Piersol (7).

Neural Crest

A strand of distinctive cells lies beneath the ectoderm between the optic and otic primordia (Figs. 1 and 2), forming a cap for the underlying mesoderm (Figs. 5 and 7, *N. cr.*). This I take to be the neural crest. Under low power (Fig. 7, *N. cr.*), the nuclei of the crest cells remind one of spermatozoa heads—oval, bead-like, dark staining nuclei with scanty cytoplasmic processes. Under high power (Fig. 17) the neural crest blends with the mesoderm. The Vant embryo is the youngest human embryo so far reported with a neural crest. Bartelmez and Evans (1) begin their study of the neural crest with the seven somite stage.

Subdivisions of the Nervous System

The identification of the otic placode enables one to establish a few areas of the future brain. The sharp bend in the neural fold halfway between the otic placode and the brain tip marks the midbrain. Between the otic placode and the first somite the neural groove is wider. At this level may be put the rhombomere "C" of Bartelmez and Evans (1). Rhombomere "B" would lie between the two otic placodes. Rhombomere "A" and the trigeminal area are still indefinite.

Fore-gut

The shallow fore-gut (Figs. 2 and 3 to 7) is tipped with a special clump of cells. The dorsal part of the clump (Figs. 3 and 16, *P.p.*) pertains to the notochord and is the prechordal plate. The ventral part (*O.m.*) is fused with the underlying ectoderm to form a very short oral membrane. The dorsal surface of the fore-gut is deeply furrowed to accommodate the neural groove (Figs. 4 to 8). No pharyngeal pouch or thyroid gland can be made out. In general

EXPLANATION OF FIGURES

FIGS. 3 TO 10. Photomicrographs of sections at levels indicated in Fig. 2. Labels as in Fig. 2. $\times 98$.

Fig. 3. Section through prechordal plate, oral membrane, and optic primordium.

Fig. 4. Section through fore-gut and vascular primordium of the heart.

Fig. 5. Section through fore-gut and heart.

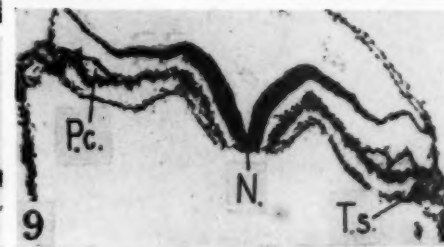
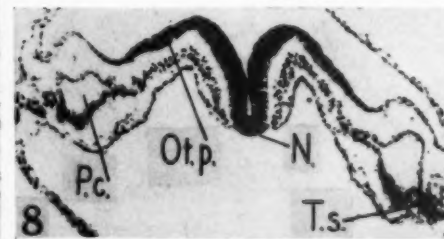
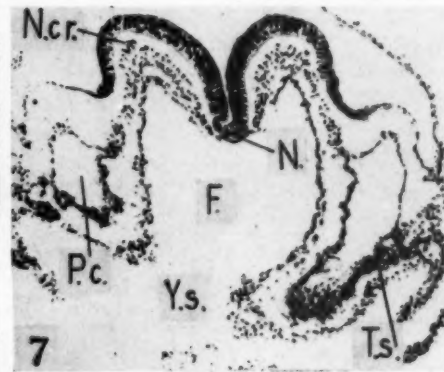
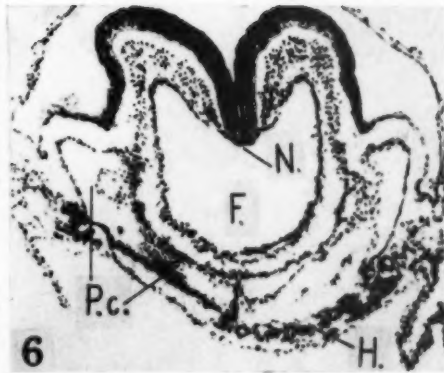
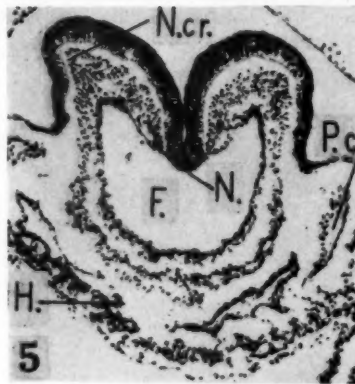
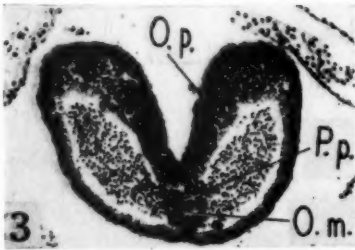
Fig. 6. Section through fore-gut, heart, and pericardial cavity.

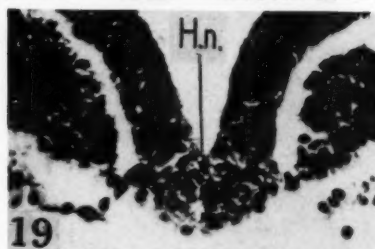
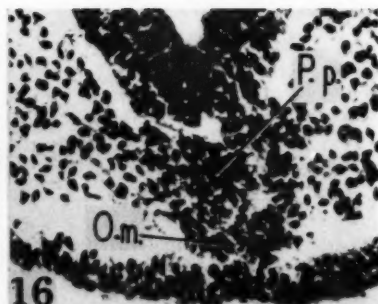
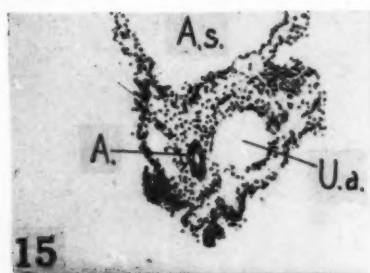
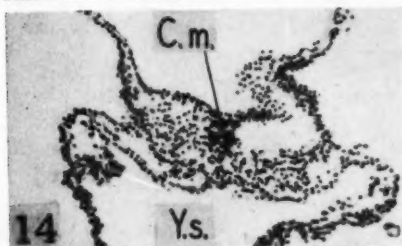
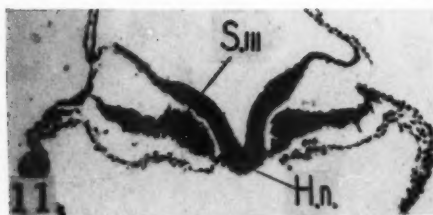
Fig. 7. Section through fore-gut entrance, body cavities, and pre-otic neural crest.

Fig. 8. Section through otic placode.

Fig. 9. Section through rhombomere "C" and tips of body cavities.

Fig. 10. Section through first somite.





the Vant embryo takes a middle place among known two to three somite embryos. The Ludwig (4) and Piersol (7) embryos have no oral membrane, pouch, or thyroid. The Wilson (11) embryo has no oral membrane but does show traces of a first pouch and thyroid. The Ingalls (3) embryo has all three. The slightly older four somite embryos of Sternberg (9) and Orts Llorca (6) have definite first pouches.

Hind-gut and Allantois

The Vant embryo has no hind-gut, although one is present in other embryos of the same age. The yolk sac sends a funnel-shaped allantoenteric diverticulum beneath the cloacal membrane (Fig. 2). From its tip extends a tubular allantois of moderate length. The allantois epithelium is thicker than that of the entoderm elsewhere (Fig. 15).

Henson's Node, Notochord, Prechordal Plate

Between the neurenteric canal and the second somite the yolk sac and the overlying ectoderm are tied together by Henson's node (Figs. 2, 11, and 19). From the node issues the notochord. The notochord is everywhere a flat ribbon inserted into the entoderm (Figs. 10 to 4). It clings to the overlying neural groove. Although continuous with the entoderm the notochord is easily distinguished from it. The notochord cells have rod-shaped nuclei (Fig. 18) and a cytoplasm that stains a little deeper with eosin. There is no trace of a notochordal canal. The notochord ends anteriorly in a clump of cells that is part oral membrane and part prechordal plate (Figs. 3 and 16). The prechordal plate blends with the head mesoderm.

The relations of Henson's node, the notochord, and the prechordal plate to one another and to the mesoderm are matters of perennial discussion. My observations on a single human embryo can hardly outweigh the authoritative and exhaustive studies of Streeter (10) on the pig. Streeter derives the notochord entirely from Henson's node and considers the node to exist for that purpose. In the Vant embryo the notochord certainly appears as if spun out of the node. Streeter is inclined to consider the prechordal plate a specialized entodermal structure and not a part of the notochord. The cells

EXPLANATION OF FIGURES

FIGS. 11 to 15. Photomicrographs at levels indicated in Fig. 2. Labels as in Fig. 2. $\times 98$.

Fig. 11. Through third somite and Henson's node.

Fig. 12. Through neurenteric canal.

Fig. 13. Through middle of primitive streak.

Fig. 14. Through cloacal membrane.

Fig. 15. Through allantois and umbilical arteries.

FIGS. 16 to 19. Photomicrographs of parts of previous figures at higher power. Labels as in Fig. 2.

Fig. 16. Portion of Fig. 3, to show prechordal plate and oral membrane. $\times 300$.

Fig. 17. Portion of Fig. 7, to show neural crest. $\times 300$.

Fig. 18. Portion of section close to Fig. 8, to show notochord. $\times 300$.

Fig. 19. Portion of Fig. 11, to show Henson's node. $\times 300$.

of the Vant prechordal plate are more like those of the entoderm than of the notochord (compare Figs. 16 and 18). Streeter derives the mesoderm almost entirely from the primitive streak and thinks the contact between the mesoderm and the node and notochord to be secondary or accidental, and that any mesodermal production from the prechordal plate is a supplementary one. In the Vant embryo the mesodermal sheets do stick to the node and notochord and nearby entoderm (Figs. 19 and 11 to 7) but there is nothing to indicate that these structures are adding to the mesoderm. On the other hand the blending of the prechordal plate with the head mesoderm is very intimate, so intimate as to make one suspect the mesoderm is receiving additions from this source. Perhaps the supplementary activity of the prechordal plate is greater in man than in the pig.

Neurenteric Canal

The neurenteric canal (Figs. 2 and 12) is included in a single section. The lumen may be plugged with cytoplasm; it is minute at any rate. The dorsal aperture is funnel-shaped and spread over the three following sections.

Primitive Streak

Behind the neurenteric canal lies the primitive streak (Fig. 2). A mid section is shown in Fig. 13. The mesoderm is everywhere streaming out from the under side of the ectoderm. The subjacent entoderm is fairly distinct. As already stated, the streak is not so well preserved and stained as other structures. It has the appearance of post-mortem fixation and staining.

Cloacal Membrane

Next behind the streak follows a long cloacal membrane. It extends the full length of the allantoenteric canal and overlaps the allantois (Fig. 2). It shows the characteristic fusion of ectoderm and entoderm (Fig. 14), although exclusion of mesoderm is not easy. The anterior third of the membrane is a continuous structure, and poorly preserved like the primitive streak. The caudal two-thirds of the cloacal membrane are perforated in several places (Fig. 2) and show numerous dark staining degeneration granules—signs of an impending shortening of the membrane. A similar degeneration of the caudal part of the cloacal membrane occurs in the four somite embryos of Sternberg (9) and Florian (2). Florian has studied this area in detail and has shown that such a degeneration is normal. Extrophy of the bladder may be traced to some irregularity at this period.

Mesoderm and Somites

The mesoderm of the Vant embryo consists of two great para-axial sheets streaming out of the primitive streak (Fig. 13). At the edge of the embryonic disk each sheet splits to envelop the amnion and yolk sac. The mesoderm extends caudally into the umbilical stalk (Fig. 15). Traced forward the two sheets are loosely attached to Henson's node (Fig. 11), and the notochord (e.g., Fig. 7). They envelop the fore-gut and fill the head fold (Figs. 6 to 3).

As Streeter states, the only really intimate connection of the mesoderm is with the primitive streak and with the prechordal plate.

There are two definite pairs of somites and the start of a third pair. The first pair lies just rostral to the union of the notochord and Henson's node (Figs. 2 and 10). The first pair shows typical radial cell arrangement and a central cavity. The second pair lacks the central cavity on the right side. Each somite of the third pair (Fig. 11) is a simple heap of cells.

Body Cavities

Just anterior to the first somite each mesodermal sheet is split to form a pericardial cavity (Figs. 9, 8, 7). The cavities communicate beneath the fore-gut (Figs. 2 and 6) through a tiny transverse passage. Each cavity then extends forward (Figs. 5 and 4) as a blind recess beneath the ectoderm. The general shape of the two pericardial cavities is that of a pair of water wings. The earlier isolated bits of body cavity have all, or nearly all, been absorbed into the general structure. The splanchnic layer beneath each pericardial cavity is thickened into a cardiogenic plate.

Vascular System and Heart

The vascular system of the Vant embryo has reached an indeterminate stage that baffles reconstruction. About two-thirds of the yolk sac is sprinkled with blood islands (Fig. 20). A large blood island on each side of the mouth of the allantois continues into a well defined umbilical artery (Figs. 20 and 15) that extends the full length of the allantois. Each vessel is a well defined

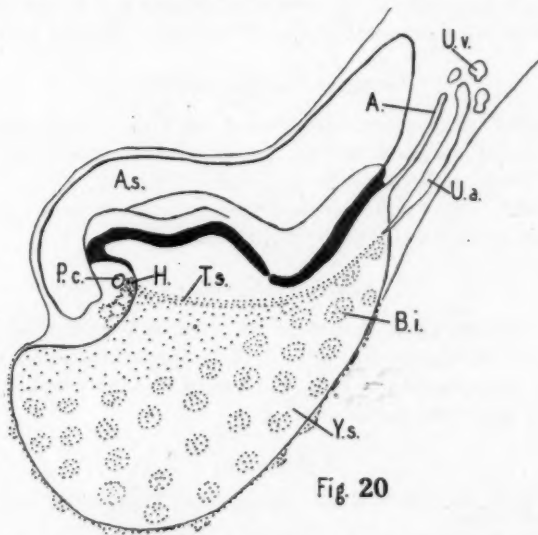


FIG. 20. Outline of entire Vant embryo to show vascular system. B.i., blood island; U.a., umbilical artery; U.v., umbilical vein.

tube containing free blood cells. Around the ends of the arteries are a cluster of irregular spaces that may be umbilical veins.

Over the anterior third of the yolk sac blood islands are replaced by a more continuous sheet of angioblastic tissue (Fig. 20), which forms a solid terminal sinus along the margin of the embryo proper (Figs. 20, 9, and 8). Each terminal sinus connects caudally with the blood islands from which the umbilical artery arises. Traced anteriorly the sinuses spread medially beneath the thickened mesoderm and then join beneath the transverse passage between the two pericardial cavities (*H* in Figs. 20, 6, 5, and 4). The spongy transverse strip of angioblastic tissue with the overlying cardiogenic thickening of the mesoderm constitutes the primordium of the heart.

There are also many traces of an angioblastic plexus inside the embryo, especially anterior to the neurenteric canal. There are spurs from the terminal sinuses and scattered cells that cling to the dorsal side of the endoderm (Figs. 10 to 5). The fore-gut has a collar of such cells, the forerunner of the first arterial arch. Scattered para-axial cells mark the course of the future dorsal aortae.

Taken as a whole, the vascular system of the Vant embryo falls between that of the presomite embryo of M'Intyre (5) and the Ludwig embryo (4). The outstanding feature of the vascular system of the Vant embryo is its sharp division into an anterior part converging on the heart and a posterior part converging on the umbilical arteries, and the decided precocity of the latter. The umbilical arteries are recognizable as such, but of the heart only source materials are present. The curious precocity of the umbilical arteries is found in most other human embryos of the same stage of growth.

General Considerations

As the detailed comparisons have shown, the Vant embryo does not depart greatly from other embryos of the two to three somite stage. The Vant embryo, and those of Ingalls (3), Ludwig (4), Piersol (7), and Wilson (11) corroborate each other and give a definite and consistent picture of the human embryo at their stage of development.

Acknowledgments

I conclude with my great thanks to Dr. J. Ross Vant for the embryo that is the subject of this paper, to Mr. A. G. Fairall who prepared the serial sections of the embryo, and to Prof. W. Rowan who made the splendid drawing of the wax model reconstruction.

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RESISTANCE TO EXTREME TEMPERATURES IN CONNECTION WITH DIFFERENT DIETS¹

BY L. P. DUGAL², C. P. LEBLOND³, AND M. THÉRIEN⁴

Abstract

The purpose of this research was to investigate the relative value of different diets for conferring on animals resistance to extremes of temperature. These diets were equicaloric and equivitaminic and differed only in the relative proportions of fats, proteins, and carbohydrates. The self-selection method of feeding was used, with rats exposed to low and to high temperatures. The results obtained with this method have been verified on large groups of animals adapted and not adapted to extreme temperatures. It has been found that a diet rich in fats is decidedly superior to one rich in carbohydrates (both diets being equicaloric and equivitaminic) for adaptation and resistance to cold on the part of the animals, and that a diet rich in carbohydrates and poor in fats is much more favourable than one rich in fats for conferring resistance to heat.

Introduction

Little experimental work has been done on the effect of diet on the resistance of animals to cold and heat (2, 3). The object of the present work was to explain possible differences in the ability of animals to resist extreme temperatures when fed *equivitaminic* and *equicaloric* diets in which only the proportions of *proteins*, *carbohydrates*, and *fats* are varied. In order to ascertain the most satisfactory diet under these conditions, the self-selection method of feeding was used. The criteria for the best diet were survival and growth of the animals. It was the writers' purpose to verify on large groups of animals the results obtained on a few individuals by this method.

Methods and Technique

(1) SELF-SELECTION METHOD OF FEEDING (1, 2, 5-11)

Six adult male white rats of the same litter were placed in large individual cages. Each cage was supplied with food cups containing, respectively, casein, glucose, lard, brewers' yeast*, cod liver oil, and wheat germ oil, and with drinking bottles containing water or various salt solutions (sodium chloride (3%), potassium chloride (1%), magnesium chloride (0.5%), mono-

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² Professor of General Physiology, Université de Montréal.

³ Assistant Professor of Histology, McGill University.

⁴ Research Assistant in General Physiology, Université de Montréal.

* The composition of yeast (Molson's) was reported to be as follows:

Protein	37.44%
Fat (ethyl ether extract)	1.70%
Carbohydrates (by difference)	50.02%
Crude fibre	2.66%

Some information was available on vitamin content; nicotinic acid, 0.51 µgm. per gm.; riboflavin, 41 µgm. per gm.; thiamin, 32 I.U. per gm.

sodium phosphate (8%), and calcium lactate (2.5%)). After the animals had been in the cage for more than two weeks, the estimation of foodstuffs and salt solutions consumed was begun and thereafter repeated every other day. Four weeks later the animals were placed in a cold room at a temperature of -2°C ., where they were kept for 60 days. The animals were then kept for 16 days in a warm room maintained at 32°C . and then for 40 days at 35°C . Finally, another set of control values was obtained by keeping the animals at room temperature (about 22°C .) for another month. In all cases, the amount of each foodstuff ingested was expressed as percentage of the total solid intake in two days. In the figures, these results were expressed as averages for periods of six days.

(2) CONFIRMATION ON LARGE GROUPS OF ANIMALS

A. At Low Temperatures

A good and a poor diet (called respectively *A* and *R*) for conferring resistance to low temperatures (as indicated by the behaviour of the rats submitted to self-selection feeding) were compared on large groups in the following four ways:

(a) *Animals of both groups (A and R) not adapted to cold nor to their new diets (A and R) before exposure to cold (-4°C .)*. Comparison of the two diets has been made on two groups of 60 animals each, one group receiving the diet rich in fats (*A*) and the other group receiving the diet rich in carbohydrates (*R*). This first set of experiments was made on animals of the same age and weighing 200 gm. on the average. The animals were then suddenly transferred from normal temperature to a temperature of -4°C . In this instance, therefore, neither group was adapted to cold, nor preadapted to their respective diets, both groups receiving a "Fox chow" diet at normal temperature, and then new diets, respectively *A* and *R*, only when they were suddenly exposed to a lethal temperature of -4°C . The criterion used in this series of experiments was the percentage of survival.

(b) *Animals of both groups preadapted to their respective diets (A and R) (fed their respective diets during two weeks at normal temperature before entering the cold room) but not adapted to cold (-4°C .)*.

This series of experiments was also made on 120 rats (60 rats for each group), and these rats, preadapted to their diets, were suddenly exposed to a lethal temperature of -4°C . Animals of both groups were of the same age and weighed about 200 gm. Here, also, the criterion used was the percentage of survival.

(c) *Animals adapted to cold and pretreated with their diets*. In this series, the effect of diet *A* (rich in fats) was determined on 30 rats exposed gradually to 4° , 2° , 0° , and -2°C ., as compared with the effect of the diet *R* (rich in carbohydrates) on another group of 30 rats gradually exposed at the same time to the same decreasing temperatures.

All rats were of the same age and weighed about 240 gm. when placed in the cold room, after an adaptation period of 15 days at room temperature to both diets.

This time, instead of taking as the criterion the percentage of survival in each group, the writers have taken growth curves as a more sensitive criterion of the more favourable diet.

(d) *Diets A and R were also tested at normal room temperature.* A few details of technique, concerning the above four series of experiments, are added here:

1. Both diets were *equicaloric* and *equivitaminic*. This was ensured by controlling the amount of food ingested every day by each rat of each group.
2. The humidity was maintained between 50 and 60%.
3. Each animal (white rat) was placed in a small individual cage, which did not allow for much exercise. Frequent observations of the animals have shown that they were at rest most of the time.

B. *At High Temperatures*

The procedure followed here was almost the same as that described for resistance to cold, except that the good (*E*) and the poor (*D*) diets (as indicated by their effect on rats submitted to self-selection feeding at high temperatures) were compared only in regard to animals adapted to their diets and gradually exposed to increasing heat.

Results and Discussion

(1) SELF-SELECTION METHOD OF FEEDING

A. *Effect of Low Temperature* (See Tables I and II, and Figs. 1-6)

All the animals but one (*R*, Fig. 6) survived the cold and were in good condition. The total food consumption of the animals was increased (Tables I and II). On the average, there was little change in the proportion of ingested casein, glucose, lard, and cod liver oil, but there was a marked decrease in yeast ingestion and a marked increase in consumption of wheat germ oil. Examination of individual results showed contrast between the food consumption of the animal that failed to withstand cold (Fig. 6) and the others, especially the one that gained much weight in the cold (Fig. 1). Three of the animals consumed little casein in their first period at room temperature; when in the cold, all but one (Fig. 6) raised their consumption of casein to more than 20% of their food intake (Tables I and II). The total consumption of fat, as obtained by adding the lard and the oils, was approximately doubled in the cold, except again in the case of the animal that died in the cold.

There was little variation in the ingestion of salts in the cold. The consumption of sodium chloride increased to a maximum during the second or third week after the beginning of exposure. The ingestion of monosodium

TABLE I

AVERAGE AMOUNT OF EACH FOODSTUFF (IN PERCENTAGES) CHOSEN BY EACH RAT

Foodstuff	Temperature	A	B	C	D	E	R
Proteins	Normal	6.14	32.5	31.5	0.56	36.72	0.71
	Low	22.33	36.14	31.42	24.28	27.6	2.94
	High	12.56	19.88		16.37	13.75	
Carbo- hydrates	Normal	35.42	39.9	37.5	43.4	32.06	61.95
	Low	32.1	45.33	37.76	35.76	44.2	82.56
	High	53.26	66.63		23.98	56.34	
Lard	Normal	4.41	2.49	4.99	10.69	15.11	3.36
	Low	10.21	4.73	4.85	6.23	4.7	0.24
	High	11.91	1.4		35.31	10.12	
Yeast	Normal	38.27	8.25	8.9	41.9	13.84	31.02
	Low	13.19	5.86	8.53	5.5	10.42	12.78
	High	13.37	6.6		13.27	7.67	
Cod liver oil	Normal	0.36	0.19	0.97	0.65	0.55	0.10
	Low	0.983	0.58	0.88	0.67	0.51	0.44
	High	1.53	1.46		1.40	1.98	
Wheat germ oil	Normal	9.5	0.4	9.5	2.05	0.34	0
	Low	20.21	10.06	14.15	25.96	12.78	0.9
	High	3.45	2.14		1.3	1.58	

TABLE II

AVERAGE AMOUNT OF EACH FOODSTUFF (IN GRAMS)

Rats	Protein	Carbo- hydrate	Fat	Yeast	Cod liver oil	Wheat germ oil	NaCl	KCl	MgCl ₂	Phos- phate	Lactate
Rat A											
Normal temp.	1.13	6.39	1.1	5.75	0.14	1.6	0.0519	0.012	0.0485	0.264	0.025
Low temp.	6	9.25	2.5	3.88	0.26	5.7	0.045	0.083	0.0826	0.152	0.0725
High temp.	2.9	5.26	1.8	1.3	0.26	0.387	0.0564	0.03	0.005	0.1312	0.8875
Rat B											
Normal temp.	9.17	9.62	0.45	2.9	0.22	0.11	0.0357	0.099	0.0548	0.2024	0.0442
Low temp.	10.2	12.5	0.69	1.6	0.16	2.4	0.0891	0.0519	0.0263	0.088	0.2125
High temp.	2.3	8.14	0.16	0.84	0.2	0.3	0.044	0.0465	0.0102	0.1016	0.0605
Rat C											
Normal temp.	5.16	6.27	0.6	1.66	0.17	1.44	0.36	0.022	0.0341	0.06	0.036
Low temp.	6.76	9.22	1.1	1.33	0.21	3.1	0.1233	0.0582	0.0215	0.144	0.0762
Rat D											
Normal temp.	0.12	8.15	2.09	7.2	0.16	0.48	0.0504	0.1604	0.0074	0.096	0.028
Low temp.	5.5	8.4	1.25	1.32	0.14	6.13	0.144	0.049	0.0145	0.028	0.0735
High temp.	1.4	2.46	2.8	0.93	0.14	0.13	0.0504	0.0293	0.0078	0.096	0.0725
Rat E											
Normal temp.	8.58	6.86	3.2	3.04	0.14	0.17	0.0273	0.0133	0.0099	0.148	0.0252
Low temp.	9.05	13.79	1.64	3.4	0.19	3.61	0.0873	0.077	0.0299	0.1192	0.03825
High temp.	1.85	6.56	1.71	1.0	0.27	0.2	0.0663	0.0228	0.0109	0.2944	0.0385
Rat R											
Normal temp.	0.19	13.2	0.78	4.89	0.07	0.107	0.036	0.1048	0.0083	0.12	0.0347
Low temp.	0.25	23.6	0.065	3.33	0.16	0.26	0.0426	0.0318	0.0133	0.024	0.0975

phosphate decreased in the cold, but that of calcium lactate increased. The amount of magnesium chloride consumed became negligible during the second month of exposure.

B. Effects of High Temperature (See Tables I and II, especially the results for E and D)

The effects of heat were detectable at 32° C. but most marked at 35° C. The total food consumption decreased. In proportion, the ingestion of

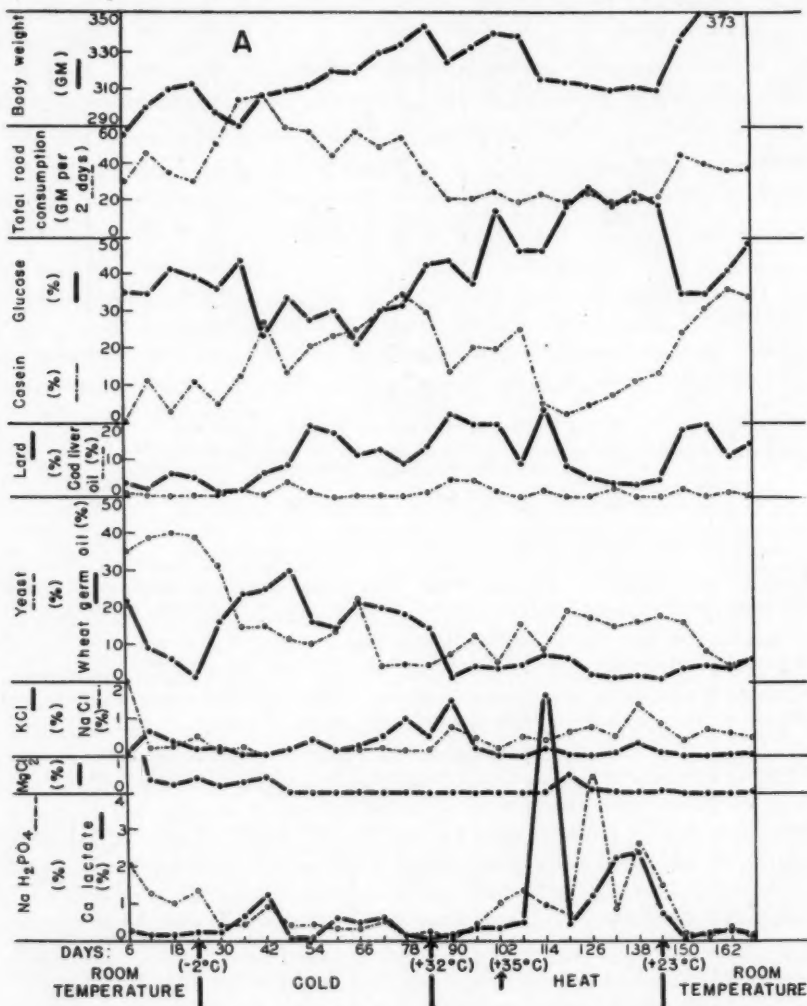


FIG. 1. Amount of each foodstuff (in percentage) chosen by Rat A at room temperature, at -2° C., and at 32° and 35° C., and also the body weight, and the total food consumption.

carbohydrates was greater, and that of casein and lard smaller, than in the cold. There was, however, one exception, since one animal consumed fairly large amounts of fats; this animal appeared to suffer from exposure to heat, and was in poor condition at the time of transfer from the temperature of the warm room to room temperature. The ingestion of wheat germ oil decreased markedly in the heat, while that of yeast increased slightly. A slight increase in the ingestion of cod liver oil was of doubtful significance. The consumption

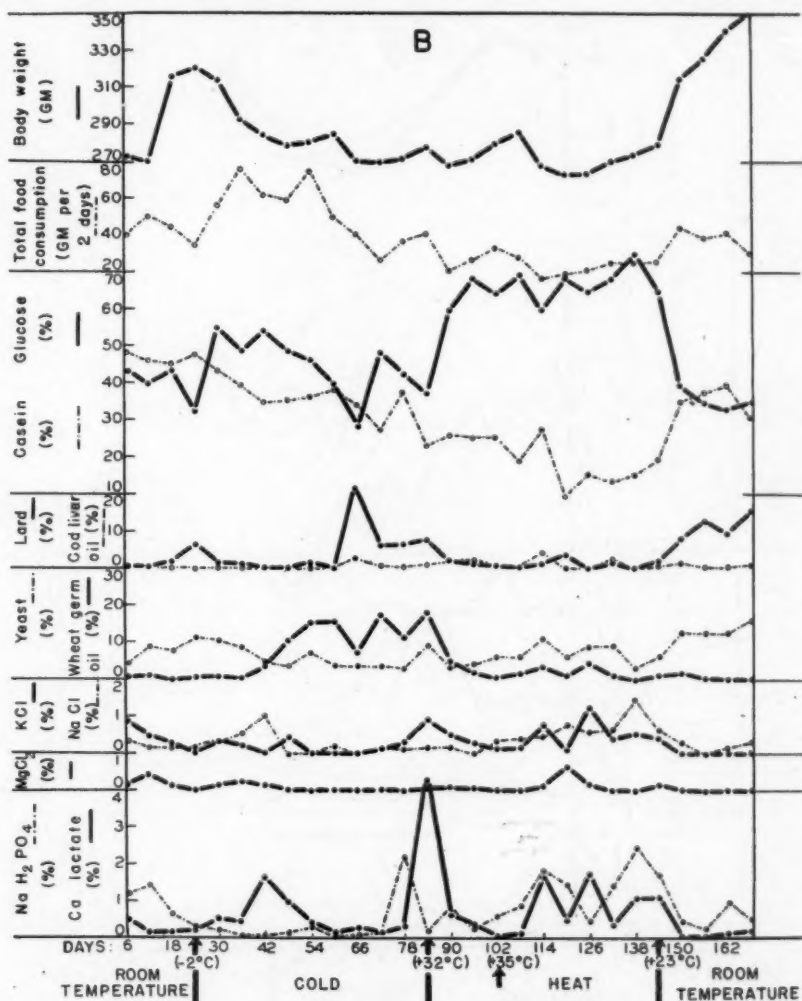


FIG. 2. Body weight and total food consumption of Rat B, at the temperatures indicated, and the amount of each foodstuff chosen by the same animal at the same temperature.

of all salts, except magnesium chloride, was increased at high temperature, the most striking change being observed in the case of the phosphate solution.

C. Discussion

If the total value of the various foodstuffs as sources of proteins, fats, or carbohydrates is considered, it may be seen that fat consumption (i.e., lard plus wheat germ oil) was markedly increased in the cold and decreased in the heat. Thus, lipids furnished about one-fourth of the caloric intake at room

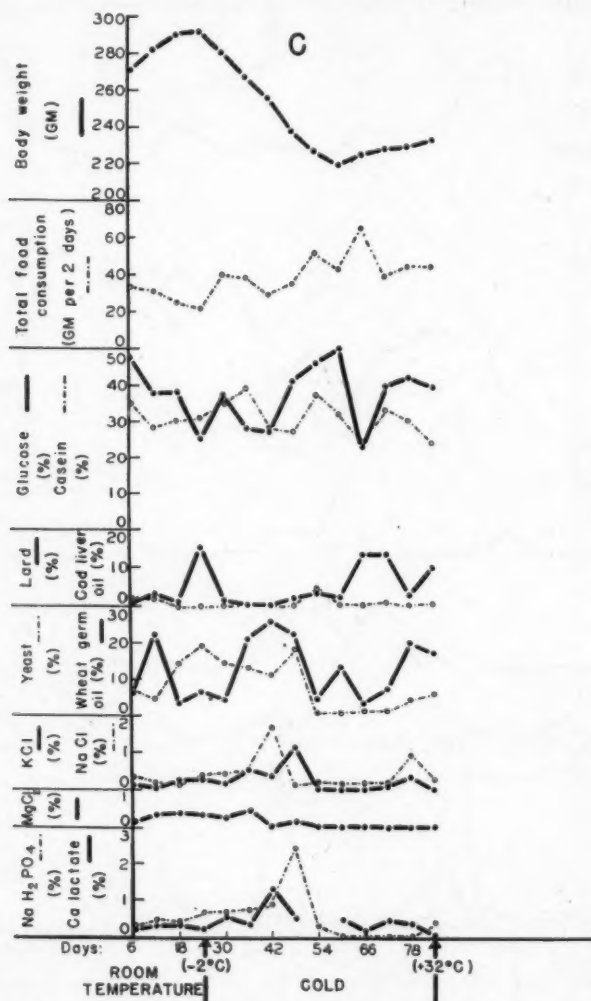


FIG. 3. Rat C at room temperature, and at -2°C .; amount of each foodstuff chosen, body weight, and total food consumption.

temperature and about one-half of it in the cold. The total consumption of proteins (casein and yeast) was not significantly changed in the cold, but it decreased markedly in the heat.

The total ingestion of carbohydrates (glucose and yeast) usually decreased in the cold. In the warm room, it was much greater than at room temperature or in the cold.

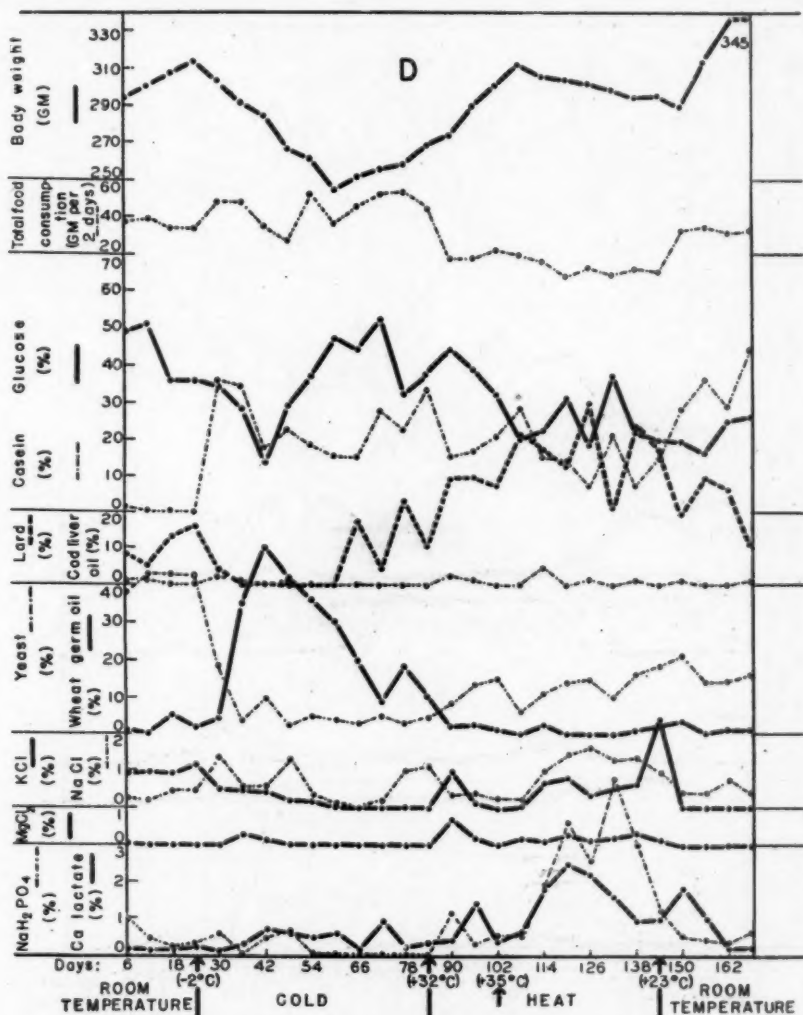


FIG. 4. Rat D at room temperature, at -2°C ., and at 32°C and 35°C .; amount of each foodstuff chosen, body weight, and total food consumption.

The shift from one source of fat or protein to another was quite noticeable. Thus, in the cold, the rats selected wheat germ oil instead of lard; this is the opposite to what happened at room temperature or in the heat. Similarly, there was an inverse relation between the consumption of casein and yeast in the cold and the warm rooms. The fact that yeast and wheat germ oil, in addition to furnishing energy materials, contain vitamins, could account for some of the changes. Again one particular type of fat or protein may be

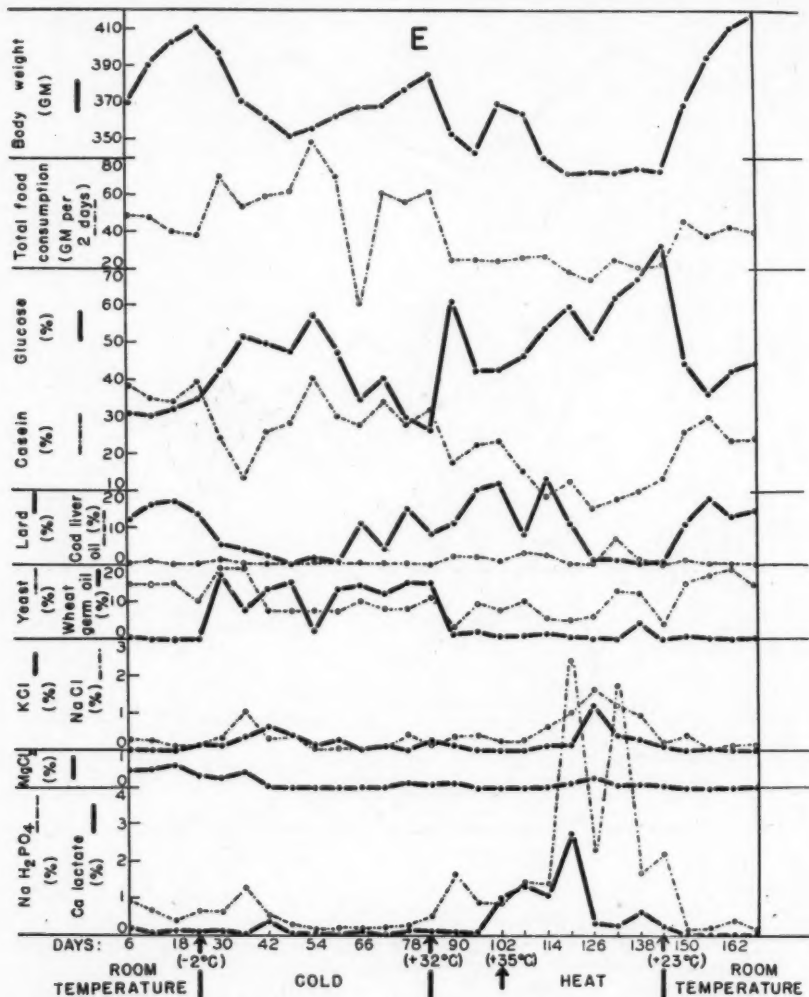


FIG. 5. Rat E at room temperature, at -2°C ., and at 32°C and 35°C .; amount of each foodstuff chosen, body weight, and total food consumption.

preferred under one or the other condition. Finally, simpler explanations such as palatability must be considered; thus the hardness of lard at 0° C. may account for the apparent substitution of wheat germ oil for lard in the cold room.

In the cold, one of the animals made food selections entirely different from those of the others, as it increased glucose consumption at the expense of other foodstuffs, taking almost no fats or casein (Fig. 6). The fact that this

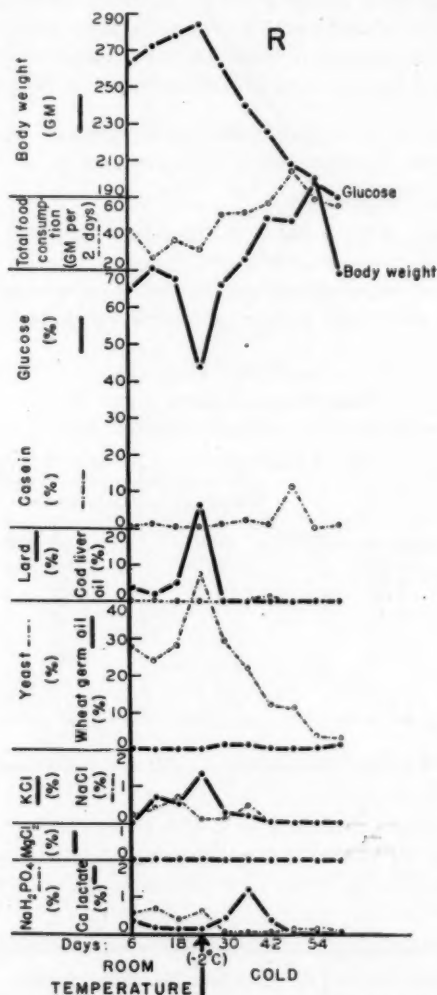


FIG. 6. Rat R at room temperature and at -2° C.; amount of each foodstuff chosen, body weight, and total food consumption.

animal died in the cold, suggested that the five others, which survived in good condition, had selected a satisfactory diet.

Similarly, the only animal to consume a large amount of fats in the warm room was in poor condition. The others, for which glucose was the main source of calories, were in good shape; this suggested that their selection of food was beneficial.

On the whole, the mineral intake was in fair proportion to the total food intake at all temperatures, except at 35° C., at which temperature the increase in salt uptake may be related to the loss of mineral elements in sweat. However, the considerable increase in ingestion of calcium and phosphate at high temperatures pointed to a more specific disturbance in their metabolism.

(2) CONFIRMATION OF THE PRECEDING RESULTS (WITH SELF-SELECTION FEEDING) ON LARGE GROUPS OF ANIMALS

A. Resistance to Low Temperatures

The two following diets, *A* being the better as found by the self-selection method, and *R* being the poorer (the one chosen by the rat that died), have been tested for their respective values in conferring resistance to cold and adaptation to cold with large groups of rats under the following conditions (see Table III).

TABLE III
COMPOSITION OF DIETS *A* AND *R*

Constituent	Diet <i>A</i> (high fat)		Diet <i>R</i> (high carbohydrate)	
	Grams	Calories	Grams	Calories
Casein	4.30	18.9	2.50	11
Dextrose	6.20	24.5	20.00	79
Lard	1.86	17.27	0.40	4.65
Yeast	1.25	8	1.25	8
Cod liver oil	0.30		0.30	
Wheat germ oil	3.71	33.93	0.03	
Mineral salts	1.00		1.00	
		Total 102.6		Total 102.6

(a) *Animals of both groups not adapted to cold, nor to their new diets, A and R, before exposure to cold (-4° C.).*

The results are summarized in Fig. 7 and show a survival of 60% after seven days for the group receiving the diet rich in fats (*A*), as compared to a survival of less than 10% for the group receiving the diet rich in carbohydrates (*R*).

(b) *Animals of both groups preadapted to their respective diets.*

The results, summarized in Fig. 8, show a survival of more than 80% after 20 days for the group receiving the diet rich in fats (*A*), as compared to a survival of about 40% for the group receiving the diet rich in carbohydrates.

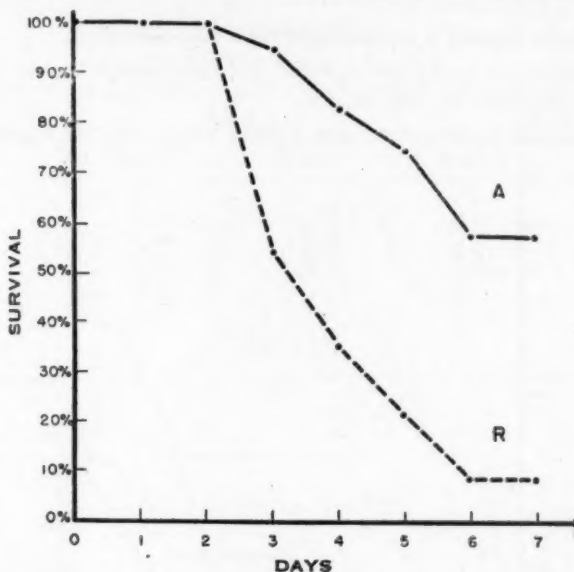


FIG. 7. Survival, at -4°C ., of animals of both groups (A and R), not adapted to cold or to their new diets.

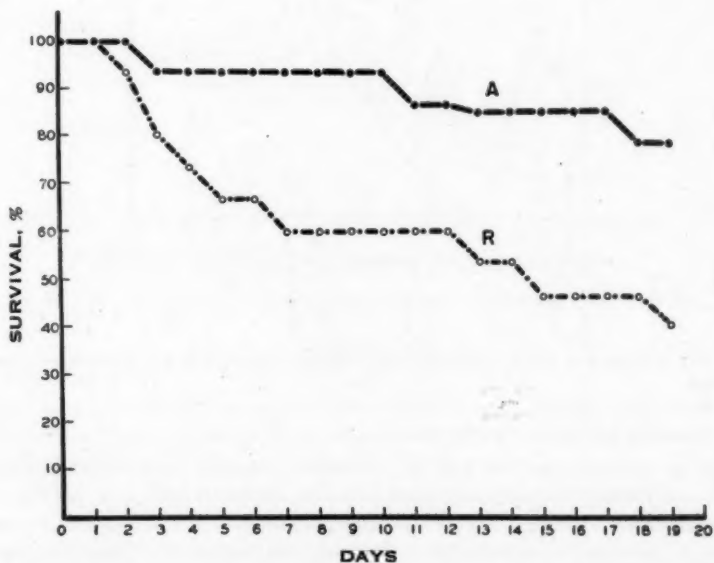


FIG. 8. Survival, at -2°C ., of animals of both groups not adapted to cold, but preadapted to their respective diets.

(c) *Animals adapted to cold and pretreated with their diets.*

The experiment lasted six months, and the growth curves for the two diets differ considerably (see Fig. 9).

(d) At normal room temperature, growth was normal with each diet.

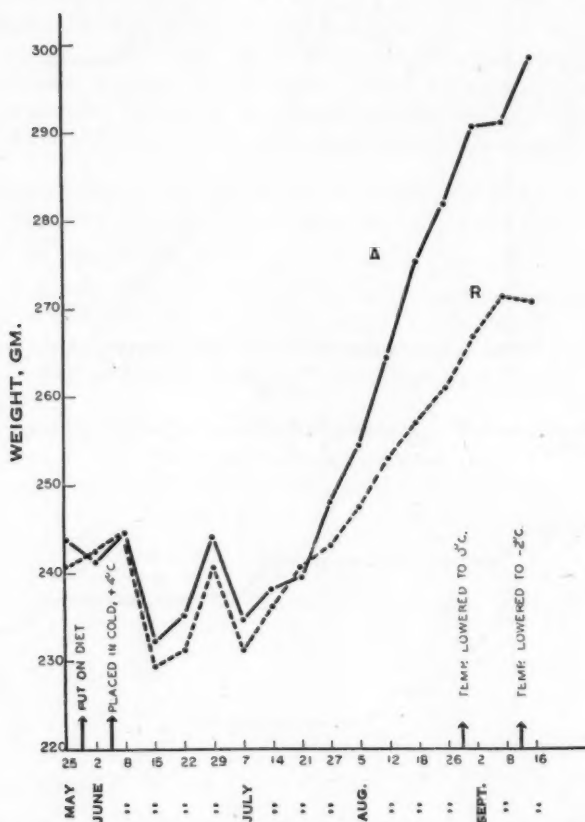


FIG. 9. Growth of animals of both groups, adapted to cold and preadapted to their respective diets.

B. Resistance to High Temperatures

As far as resistance to heat is concerned, the best diet according to self-selection feeding experiments is *rich in carbohydrates and poor in fats*. This was confirmed by verifying the results obtained in the self-selection method on large groups of animals (Diet E being the better, D being the poorer—see Table IV.)

The results were obtained with 120 animals and are expressed in Fig. 10.

TABLE IV
COMPOSITION OF DIETS E AND D

Constituent	Diet E		Diet D	
	Grams	Calories	Grams	Calories
Casein	2.6	11.4	3.1	13.6
Glucose	15.6	61.6	5	19.7
Lard	2.4	20.5	6.6	61.4
Yeast	1.25	8.0	1.25	8.0
Cod liver oil	0.3		0.3	
Wheat germ oil	0.03		0.03	
	Total 101.5		Total 102.7	

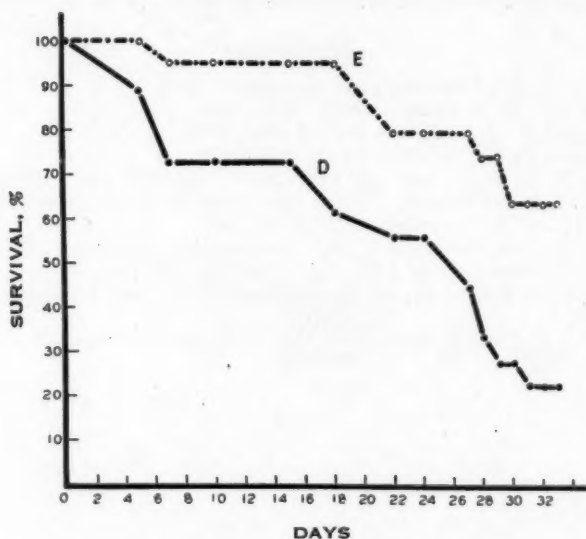


FIG. 10. Survival, at 35° C., of animals receiving respectively diets E and D.

Conclusions

1. Self-selection

(a) Rats offered an assortment of foodstuffs were usually able to select a diet that is necessary for survival in good condition at normal (20 to 25° C.), low (0 to -2° C.), or high (32° to 35° C.) temperature.

(b) About one-fourth of the food consumed in the cold was fat; another fourth was casein. The only animal that did not increase its consumption of casein and fat succumbed at this temperature.

(c) In the heat (35° C.), two-thirds of the food consumed was glucose; almost no fats were ingested.

(d) The animals selected a greater amount of salts at high temperatures, especially calcium lactate and monosodium phosphate.

2. Confirmation on Large Groups

The best and the poorest diets (among those tried) for conferring resistance to cold and heat (as indicated by the self-selection feeding results) were verified on large groups of rats; from all the results obtained it is concluded that:

(a) A diet rich in fats is decidedly superior to one rich in carbohydrates (both diets being equicaloric and equivitaminic, the amount of food ingested by each rat daily being taken into account) for adaptation and resistance to cold.

(b) As far as resistance to heat is concerned, the best diet must be rich in carbohydrates and poor in fats, both diets being equicaloric and equivitaminic.

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SECTION E



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1911

1911

1911

1911

1911

1911

1911

1911

SECTION E
INDEX TO VOLUME 23

Authors

- Allmark, M. G.**—See Bachinski, W. M.
- Anderson, R., Orr, J. H., and Reed, G. B.**—Influence of sulphonamides on fibroblasts, 80.
- Bachinski, W. M., Allmark, M. G., and Morrell, C. A.**—An improved procedure for the bio-assay of pituitary extract (posterior lobe), 126.
- Barberie, M.**—See Hoar, W. S.
- Bateman, J. B. and Lang, J.**—Formation and growth of bubbles in aqueous solutions, 22.
- Benham, G. H.**—The fate of phenothiazine in rabbits. I. The detection of a new conjugate in rabbits' urine after the feeding of phenothiazine, 71.
- Blanchaer, M. C.**—See Boyd, E. M.
- Boyd, E. M. and Blanchaer, M. C.**—The effect of potassium iodide, sodium iodide, and Iod-Ethamine upon the concentration of alcohol-soluble and alcohol-insoluble fractions of blood iodine, 206.
- Boyd, E. M., Blanchaer, M. C., Copeland, J., Jackson, S., Phin, K., and Stevens, M.**—The effect of inorganic and organic iodides upon the output of respiratory tract fluid, 195.
- Cameron, A. T.**—The relative sweetness of various sweet compounds and of their mixtures, 139.
- Cameron, A. T. and Guthrie, J. S.**—Determination of bromide-bromine in the blood of patients undergoing treatment with bromide, 41.
- Cameron, A. T., Meltzer, S., and Lederman, J. M.**—A study of the effect of certain dietary factors on the production of tar-carcinoma in mice, 50.
- Cantor, M. M.**—See Tuba, J.
- Copeland, J.**—See Boyd, E. M.
- Craigie, J.**—Application and control of ethyl-ether-water interface effects to the separation of rickettsiae from yolk sac suspensions, 104.
- Crampton, E. W. and Mills, M. F.**—The effect of heating, the presence of antioxidants, and the level and melting point of the fat component on the nutritional value of diets as indicated in rat feeding tests, 131.
- Dugal, L. P.**—See Ferguson, J. K. W.
- Dugal, L. P., Leblond, C. P., and Thérien, M.**—Resistance to extreme temperatures in connection with different diets, 244.
- Dunlop, A. P.**—See Jaques, L. B.
- Ferguson, J. K. W. and Dugal, L. P.**—Respiratory quotients of expired and alveolar air at normal and reduced barometric pressures, 32.
- Guthrie, J. S.**—See Cameron, A. T.
- Halst, R. E.**—See Hamilton, J. I. and Irish, U.
- Hamilton, J. I.**—See Irish, U.

- Hamilton, J. I. and Haist, R. E.**—Studies on experimental shock in dogs, 89.
- Hoar, W. S. and Barberie, M.**—Distribution of riboflavin in fresh and processed fish, 8.
- Hunter, G.**—See Tuba, J.
- Irish, U., Hamilton, J. I., Haist, R. E., and Jaques, L. B.**—The prothrombin levels of animals in shock, 119.
- Jackson, S.**—See Boyd, E. M.
- Jaques, L. B.**—See Irish, U.
- Jaques, L. B. and Dunlop, A. P.**—The effect of phthalic acid on the prothrombin time of dicumarol-treated dogs, 167.
- Kastelic, J.**—See Tuba, J.
- Lang, J.**—See Bateman, J. B.
- Leblond, C. P.**—See Dugal, L. P.
- Lederman, J. M.**—See Cameron, A. T.
- Meltzer, S.**—See Cameron, A. T.
- Mills, M. F.**—See Crampton, E. W.
- Morrell, C. A.**—See Bachinski, W. M.
- Noble, R. L.**—
Observation on various types of motion causing vomiting in animals, 212.
Methods of assaying motion sickness preventives on dogs, 226.
- Orr, J. H.**—See Anderson, R. and Reed, G. B.
- Phin, K.**—See Boyd, E. M.
- Reed, G. B.**—See Anderson, R.
- Reed, G. B. and Orr, J. H.**—N¹-Benzoyl-sulphanilamide in experimental gas gangrene, 85.
- Rice, H. V.**—A suction type of electrode for electroencephalography, 19.
- Shaner, R. F.**—A human embryo of two to three pairs of somites, 235.
- Stavraky, G. W.**—The effects of oxygen on the circulatory system in conditions of anoxia and asphyxia, 175.
- Stevens, M.**—See Boyd, E. M.
- Thérien, M.**—See Dugal, L. P.
- Tuba, J., Cantor, M. M., and Hunter, G.**—Bio-assay of vitamin C in rose hips, 1.
- Tuba, J., Hunter, G., and Kastelic, J.**—Approximate nutrient composition of dried rose hips, 5.
- Ziegler, J. A.**—Use of the benzidine staining method for the study of capillaries in the cornea, 115.

SECTION E
INDEX TO VOLUME 23

Subjects

Air, Expired and alveolar, Respiratory quotients of, at normal and reduced barometric pressures, 32.

Animals

Benzidine method of staining capillaries in cornea of different species of, 115.

Cats

Effect of inorganic and organic iodides upon the output of respiratory tract fluid of, 195.

Observations on various types of motion causing vomiting in, 212.

Circulatory system of, Effect of oxygen on, in conditions of anoxia and asphyxia, 175.

Dogs

Dicumarol-treated, Effect of phthalic acid on prothrombin time of, 167.

Experimental shock in,

Effects of, 90.

Method of producing, 89.

in shock, Clotting time, plasma time, and prothrombin time in, 120.

Methods of assaying motion sickness preventives on, 226.

Observations on various types of motion causing vomiting in, 212.

in shock, Prothrombin levels of, 119.

Mice, Effect of dietary factors on production of tar-carcinoma in, 50.

Rabbits

Effect of inorganic and organic iodides upon the output of respiratory tract fluid of, 195.

Effect of potassium iodide, sodium iodide, and Iod-Ethamine upon the concentration of alcohol-soluble and alcohol-insoluble fractions of blood iodine, 206.

urine, Detection of a new conjugate in, after the feeding of phenothiazine, 71.

Rats

Benzidine staining method for study of capillaries in cornea of, 115.

Effect of heating, presence of antioxidants, and level and melting point of fat component on nutritional value of, in rat feeding tests, 131.

in shock, Prothrombin determinations in, 122.

Resistance of, to extreme temperatures in connection with different diets, 244.

Anoxia, Effect of oxygen on circulatory system of animals in condition of, 175.

Anthelmintics, See Phenothiazine.

Antioxidants, Effect of, on nutritional value of diets in rat feeding tests, 131.

Ascorbic acid in rose hips, Bio-assay of, 1.

Ash content of dried rose hips, 5.

Asphyxia, Effect of oxygen on circulatory system of animals in condition of, 175.

Azochloramide, Effect of, on growth of fibroblasts, 83.

Barometric pressure, Normal and reduced, Respiratory quotients of expired and alveolar air at, 32.

Benzidine staining method for study of capillaries in the cornea, 115.

N¹-Benzoyl-sulphanilamide

Effect of, on growth of fibroblasts, 81.

in experimental gas gangrene, 85.

Bio-assay

of pituitary extract (posterior lobe), An improved procedure for, 126.

of vitamin C in rose hips, 1.

Blood

Effect of oxygen on the circulatory system in conditions of anoxia and asphyxia, 175.

Effect of phthalic acid on the prothrombin time of dicumarol-treated dogs, 167.

equilibrated with nitrogen or nitrogen and carbon dioxide, Evolution of gas from, 29.

of animals in shock, Prothrombin levels of, 119.

Blood iodine of rabbits, Concentration of alcohol-soluble and alcohol-insoluble fractions of, as affected by potassium iodide, sodium iodide, and Iod-Ethamine, 206.

Blood pressure changes in animals during anoxia, asphyxia, and oxygen administration, 175.

Blood vessels in cornea, Benzidine staining method for study of, 115.

Brain, See Encephalography.

- Bubbles**, Formation and growth of, in aqueous solutions, 22.
- Cancer**, Effect of diet on production of, in mice, 50.
- Capillaries** in cornea, Benzidine staining method for study of, 115.
- Carbohydrates**, Effect of, in dried rose hips, 5.
on resistance of rats to cold and to heat, 244.
- Carbon dioxide**, Evolution of, from blood, kerosene emulsion, and sodium bicarbonate solution, 29.
- Cats**, See under Animals.
- Circulatory system** of animals, Effect of oxygen on, in conditions of anoxia and asphyxia, 175.
- Clostridium novyi**, *C. septicum*, *C. sordellii*, and *C. welchii* experimental infections, N¹-Benzoyl-sulphanilamide in retardation of, 85.
- Cold**, Effect of diets on resistance of rats to, 244.
- Colloidal solutions**, Aqueous, Formation of bubbles in, 22.
- Conjugate**, New, Detection of, in rabbits' urine after feeding of phenothiazine, 71.
- Cornea**, Benzidine staining method for study of capillaries in, 115.
- Dicumarol** treatment of dogs, Effect of phthalic acid after, 167.
- Diets**
Effect of heating, presence of antioxidants, and level and melting point of fat component on nutritional value of, in rat feeding tests, 131.
Effect of, on production of tar-carcinoma in mice, 50.
Resistance to extreme temperatures in connection with, in rat feeding tests, 244.
- Digestibility** of fats of low and of high melting point, 131.
- Dogs**, See under Animals.
- Electrode**, Suction type of, for electroencephalography, 19.
- Electroencephalography**, A suction type of electrode for, 19.
- Embryo**, Human, of two to three pairs of somites, 235.
- Encephalography**, Electro-, A suction type of electrode for, 19.
- Ethyl-ether-water interface effects**,
Application and control of, to the separation of rickettsiae from yolk sac suspensions, 104.
- Eye**, Benzidine staining method for study of capillaries in cornea of, 115.
- Fat**
component, Level and melting point of, Effect on nutritional value of diets in rat feeding tests, 131.
composition of dried rose hips, 5.
in diets of rats, Effect on resistance to cold and to heat, 244.
- Fatty acids**, Unsaturated, in diet of mice, Effect of, on development of tar-carcinoma, 64.
- Feeding**
of phenothiazine, Detection of a new conjugate in rabbits' urine after, 71.
tests, See Diets.
- Fibroblasts**, Effect of sulphonamides on, 80.
- Fish**
Comparison of riboflavin content of meat and, 16.
Effect of method of processing of, on riboflavin content, 15.
Fresh and processed, Distribution of riboflavin in, 8.
- Food**
Nutrient composition of dried rose hips, 5.
See also Diets, Fish, Fruit, and Vitamins.
- Fruit** of roses, Dried,
Approximate nutrient composition of, 5.
Bio-assay of vitamin C in, 1.
- Gangrene**, **Gas**, Experimental, Sulphonamides in retardation of, 87.
- Gas**, Evolution of, from solutions, 22.
- Gas gangrene**, Experimental, N¹-Benzoyl-sulphanilamide in, 85.
- Growth** of bubbles, Formation and, in aqueous solutions, 22.
- Heat**
Effect of diets on resistance of rats to, 244.
Effect of, on nutritional value of diets in rat feeding tests, 131.
- Human embryo** of two to three pairs of somites, 235.

Hydrogen ion concentration, Effect of, on emulsion formation and separation in removal of rickettsiae from yolk sac suspensions by means of ethyl-ether-water interface, 108, 109.

Hypoprothrombinemia of dicumarol-treated dogs, Effect of phthalic acid on, 167.

Infection, Experimental gas gangrene, Sulphonamides in retardation of, 85.

Interface effects, Ethyl-ether-water, Application and control of, in separation of rickettsiae from yolk sac suspensions, 104.

Iod-Ethamine, See Iodides.

Iodides, Inorganic and organic,

Effect of, on the output of respiratory tract fluid, 195.

Inorganic iodides, 197.

Iodized proteins, fats, fatty acids, and oils, 198.

Mechanism of action of the iodides, 201.

Organic iodides, 201.

Output of iodine in the respiratory tract fluid, 205.

Effect of potassium iodide, sodium iodide, and Iod-Ethamine upon the concentration of alcohol-soluble and alcohol-insoluble fractions of blood iodine, 206.

Kerosene emulsion, Evolution of gas from, 29.

Meat, Comparison of riboflavin content of fish and, 16.

Mice, Effect of dietary factors on production of tar-carcinoma in, 50.

Molluscs, Distribution of riboflavin in, 11.

Motion, See Motion sickness.

Motion sickness

Observations on various types of motion causing vomiting in animals, 206.

Preventives, Method of assaying, on dogs, 226.

Movement, See Motion sickness.

Nitrogen, Evolution of, from blood, kerosene emulsion, and sodium bicarbonate solution, 29.

Nutrition

Nutrient composition of dried rose hips, Approximate, 5.

See also Diets, Fish, Fruit, and Vitamins.

Oxygen, Effects of, on the circulatory system in conditions of anoxia and asphyxia, 175.

in experimental animals, 177.

in humans, 186.

Phenothiazine, Fate of, in rabbits. I. The detection of a new conjugate in rabbits' urine after the feeding of thiazine, 71.

Phthalic acid, Effect of, on prothrombin time of dicumarol-treated dogs, 167.

Pituitary extract (posterior lobe), An improved procedure for the bio-assay of, 126.

Plasma, See Blood.

Potassium iodide, See Iodides.

Pressure(s)

Barometric, Normal and reduced, Respiratory quotients of expired and alveolar air at, 32.

Evolution of gas from aqueous solutions at different, 22.

Preventives for motion sickness, Methods of assaying, on dogs, 226.

Processing of fish, Effect of methods of, on riboflavin content, 15.

Proteins

in diet of mice, Effect of, on development of tar-carcinoma, 56.

in diets of rats, Effect of, on resistance to cold and to heat, 244.

in dried rose hips, 5.

Prothrombin

levels of animals in shock, 119.

time of dicumarol-treated dogs, Effect of phthalic acid on, 167.

Rabbits, See under Animals.

Rats, See under Animals.

Resistance of rats to extreme temperatures, Effect of diets on, 244.

Respiratory quotients of expired and alveolar air at normal and reduced barometric pressures, 32.

Respiratory tract fluid

Appearance of iodine-containing substances in, discussed in relation to concentrations of blood iodine, after iodine therapy, 206.

Effect of inorganic and organic iodides on output of, 195.

Riboflavin, See under Vitamins.

Rickettsiae, Application and control of ethyl-ether-water interface effects to the separation of, from yolk sac suspensions, 104.

Effect of hydrogen ion concentration, 108.

Rosa, See Rose hips.

Rose hips

Bio-assay of vitamin C in, 1.

Dried, Approximate nutrient composition of, 5.

Shock

Experimental, in dogs,

Effect of, 90.

Method of producing, 89.

Prothrombin levels of animals in, 119.

Sickness, Motion, See Motion sickness.

Sodium bicarbonate, See under Solutions.

Sodium iodide, See Iodides.

Sodium phthalate, Effect of, on prothrombin time of dicumarol-treated dogs, 170.

Solutions equilibrated with nitrogen or with nitrogen and carbon dioxide, Evolution of gas from,

Blood, 29.

Emulsion of kerosene in 0.5% Aerosol, 29.

Sodium bicarbonate, 29.

Staining of capillaries in the cornea with benzidine, Method for, 115.

Sulphadiazine

Effect of, on growth of fibroblasts, 81.

in retardation of experimental gas gangrene, 87.

Sulphanilamide

Effect of, on growth of fibroblasts, 81.

in retardation of experimental gas gangrene infection, 87.

N¹-Benzoyl-

Effect of, on growth of fibroblasts, 81.

in experimental gas gangrene, 85.

Sulphapyrazine, Effect of, on growth of fibroblasts, 81.

Sulphapyridine in retardation of experimental gas gangrene infection, 87.

Sulphathiazole

Effect of, on growth of fibroblasts, 81.

in retardation of experimental gas gangrene, 87.

Sulphonamides, Effect of, on fibroblasts, 80.

Sweet compounds and their mixtures, Relative sweetness of, 139.

Sweetness, Relative, of various sweet compounds and of their mixtures, 139.

Tar-carcinoma in mice, Effect of dietary factors on, 50.

Temperature(s), Extreme, Resistance of rats to, in connection with different diets, 244.

Toxicity of sulphonamides for tissue cells, 80.

Vascular system in cornea, Benzidine staining method for study of, 115.

Vitamins

in dried rose hips, 6.

Riboflavin

content of fish, Effect of method of processing on, 15.

content of meat and fish, Comparison of, 16.

Distribution of,

in fresh and processed fish, 8.

in molluscs, 11.

Vitamin C in rose hips, Bio-assay of, 1.

Vitamin K, Effect of, on prothrombin times of dicumarol-treated dogs, 168.

Vomiting, See Motion sickness.

Water-ethyl-ether interface effects,

Application and control of, to the separation of rickettsiae from yolk sac suspensions, 104.

Yolk sac suspensions, Application and control of ethyl-ether-water interface effects to the separation of rickettsiae from, 104.

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